

Isolation, Characterization, and
Identification of Microorganisms from
Laboratory and Full-Scale Landfills

Cincinnati Univ., OH

Prepared for

Municipal Environmental Research Lab.
Cincinnati, OH

Jul 84



U.S. Department of Commerce
National Technical Information Service

NTIS

PB84-212737

EPA-600/2-84-119
July 1984

ISOLATION, CHARACTERIZATION, AND IDENTIFICATION OF MICROORGANISMS FROM
LABORATORY AND FULL-SCALE LANDFILLS

by

J. A. Donnelly
P. V. Scarpino
University of Cincinnati
Cincinnati, Ohio 45221

Grant No. R804733-020

Project Officer

Naomi P. Barkley
Solid and Hazardous Waste Research Division
Municipal Environmental Research Laboratory
Cincinnati, Ohio 45268

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U. S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA (Please read instructions on the reverse before completing)		
1. REPORT NO. EPA-600/2-84-119	2.	3. RECIPIENT'S ACCESSION NO. PDB 4 212737
4. TITLE AND SUBTITLE ISOLATION, CHARACTERIZATION, AND IDENTIFICATION OF MICROORGANISMS FROM LABORATORY AND FULL-SCALE LANDFILLS		5. REPORT DATE July 1984
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Jean A. Donnelly and Pasquale V. Scarpino		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Civil & Environmental Engineering University of Cincinnati Cincinnati, OH 45221		10. PROGRAM ELEMENT NO. BRD1A
		11. CONTRACT/GRANT NO. Grant No. R804733
12. SPONSORING AGENCY NAME AND ADDRESS Municipal Environmental Research Laboratory--Cin., OH Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio 45268		13. TYPE OF REPORT AND PERIOD COVERED Final Report - 9/77-9/82
		14. SPONSORING AGENCY CODE EPA/600/14
15. SUPPLEMENTARY NOTES Naomi P. Barkley, Project Officer 513/684-7875		
16. ABSTRACT Studies were conducted to determine whether solid wastes in landfills contain human pathogens and whether these organisms survive landfill conditions and drain out with the leachate. Pilot-scale and commercial-sized landfill operations were evaluated, along with laboratory-sized lysimeters containing municipal solid waste, hospital waste, and sewage sludge. Total and fecal coliforms in initial leachate tended to decrease rapidly with time, whereas the numbers of fecal streptococci decreased at a slower rate. These indicator organisms were generally below detectable limits in leachates after a year, but, specific microorganisms, (including pathogens) could be isolated from these leachates and from the solid wastes. ←		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 500
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

Reproduced from
best available copy.



DISCLAIMER

Although the information described in this document has been funded wholly or in part by the United States Environmental Protection Agency through assistance agreement number R804733-020 to Pasquale V. Scarpino, it has not been subjected to the Agency's required peer and administrative review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution, and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and is a most vital communications link between the researcher and the user community.

This research provides information on the isolation and identification of chiefly bacterial pathogens and bacterial fecal microorganisms from solid waste and landfill leachate. Their survival indicates the potential for subsequent environmental pollution. Improvements in quantitative procedures for isolating bacterial pathogens in leachate aided the investigation and made possible the isolation of greater numbers and types of diverse microorganisms in samples of leachate than in previous investigations.

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory
Cincinnati

ABSTRACT

With increasing amounts of municipal and commercial solid waste entering landfills, the potential exists for microorganisms in these wastes being leached out by rainwater and contaminating ground and surface waters. To determine if the solid wastes contain human pathogens and if these pathogens survive landfill conditions and drain out with the leachate, several landfill studies were conducted.

Pilot and commercial size landfill operations were evaluated, along with laboratory size lysimeters containing municipal solid waste, hospital waste, and sewage sludge. Total and fecal coliforms in initial leachate tend to decrease rapidly with time, while the numbers of fecal streptococci decrease at a slower rate. These indicator organisms generally are below detectable limits in leachates after a year. However, specific microorganisms, including pathogens, can be isolated from these leachates as well as from the solid wastes themselves.

CONTENTS

Foreword	iii
Abstract	iv
Acknowledgements	vi
I. Introduction and Objectives	1
II. Literature Review	9
III. Landfills, Lysimeters and Other Equipment Used	82
IV. Experimental Methods.....	129
V. Results: The Development of Methods	214
VI. Results and Discussion: Simulated Landfills	255
VII. Results and Discussion: Commercial Full-Scale and Experimental Full-Scale Landfill Assays	299
VIII. Conclusions	347
IX. Recommendations	351
X. Bibliography	353
XI. Appendices	375

ACKNOWLEDGEMENT

The authors acknowledge with gratitude the involvement and assistance of many individuals, but especially Mr. Dirk Brunner, our former EPA project officer. Dirk's vision and skill guided us throughout this project and led to its successful completion. Our special thanks to Mrs. Edna McKay, who provided the technical skills required by these studies. Her devotion to the maintenance of the laboratory lysimeter is acknowledged with deep gratitude.

CHAPTER I

INTRODUCTION AND OBJECTIVES

A. INTRODUCTION

1. Discussion of the Problem

The discarded waste placed in landfills contain all of the unwanted material from a nearby population, some of which is infectious. The types of wastes placed in landfills is designated by law. The Solid Waste Disposal Act of 1965 defines solid waste as garbage, refuse, and other discarded waste, resulting from industrial, commercial, and agricultural operations. It does not include materials in domestic sewage or other pollutants in water resources, such as industrial waste water effluents, material in irrigation return flows or other common water pollutants. In actual practice sewage solids, hospital wastes, and industrial wastes are deposited along with municipal solid waste, into landfills. Some other specific examples of these deposited wastes are: soiled baby's diapers, household pet's feces, slaughterhouse refuse, dead animals, water treatment sludges, and sewage or wastewater sludge. All of these wastes are of public health importance as they may contain microorganisms pathogenic to man. The sanitary landfill with its daily soil cover is supposed to hide the unsightly trash of civilization and prevent the transfer of infectious material to

man by rats, flies, other insects, domestic and wild animals, and birds to human environmental areas. Some of the landfill contents on occasion may be washed out from the landfill by rain water, snow, and ground water. That these landfill contents may then be transported from the landfill to landsurfaces, and emptied into the ground and surface waters, is a public health concern.

Landfill use in the future is expected to increase because the amount of refuse in the United States is increasing (Niessen and Chansky, 1970). Brunner and Keller (1972) stated that in 1920 the urban areas of the United States collected only 2.75 lb of waste per capita per day, whereas in 1970 this amount increased to 5 lbs. Furthermore, they predicted that the per capita per day figure would reach 8 lbs. by 1980. They also noted that the United States produced 250 million tons of residual, commercial, and municipal wastes in 1969, although only 190 million tons was collected. In addition, they suggested that the missing portion of this waste probably went to backyards or vacant lots or were scattered across the countryside. Tchobanoglous et al. (1977) indicated that the total solid waste in the United States would rise from 170 million tons in 1980 to 230 million tons in 1990, assuming a 3.5% annual increase in waste as might be expected from the waste amounts rendered in the 1960's. This increase indicates that a large number

of newly constructed landfills will be needed.

One of the main concerns held by those who have engaged in landfill research is whether or not pathogenic microorganisms are able to survive landfill conditions, leave the landfill in the leachate, drain down through the soil, and contaminate the ground water below, which would bring about infections among populations in contact with this water (Engelbrecht et al., 1974; and Cooper et al., 1974). Also, the use of this water for drinking purposes without purification, would create a public health problem. Concerning the public health aspects of solid wastes, Hanks (1967) stated that the literature fails to support a quantitative estimate of any solid waste/disease relationship. However, the information presented does support a conclusion that, to some diseases, solid wastes has a definite, etiologic relationship. These diseases are infectious in nature. There is no similar relationship for noncommunicable disease agents in the solid wastes, not because of any negating data, but because of the lack of data. The communicable disease agents most incriminated are those found in fecal wastes especially human fecal wastes. Where these wastes are not disposed of in a sanitary manner, the morbidity and mortality rates from fecal-borne diseases in the populations are high. The conclusion is that the continued presence in the environment of the wastes themselves is the

basic causative factor. Therefore transmission - whether by direct contact, vector transfer, or indirect contact - is due to environmental contamination by such wastes.

Most investigators have concluded that these fecal bacteria and viruses do not survive landfill conditions in significant concentrations for long periods of time. If they should survive probably could not filter down through the soil to the ground water below. (Engelbrecht et al., 1974; Cooper et al., 1974; Sobsey et al., 1975; Glotzbecker, 1974; Novello, 1974, and Peterson, 1971). In a recent review, Ware (1980) stated that pathogens may survive landfill conditions and reach the ground water and surface waters. But, she indicated that a significant decrease in the viral and bacterial content does occur due to the high temperatures and the toxic action of the leachate. Although there were no studies available which documents the possibility of pathogen survival in anaerobically digested municipal solid waste, she did state that perhaps bacteria, viruses, and parasites could have survived sewage sludge digestion.

However, there is evidence for bacterial survival in the soil. Studies related to landfill deposition, such as the land application of wastes, have demonstrated the survival and travel of microorganisms in ground water. Keswick and Gerba (1980) in their review concluded that the new virus techniques have been useful in demonstrating the sur-

vival and travel of viruses in ground water. Their results indicated that microorganisms, particularly viruses, can filter down through the soil and travel 400 feet laterally in ground water from the microbial source. Their review listed the factors governing virus survival and virus mobility in ground water. Finally, the survival and lateral movement of microorganisms in ground water is supported by the work of Craun and McCabe (1973) who showed that bacteria, viruses and parasites (and a small number of chemical poisonings responsible for 3% of the outbreaks) are responsible for 57% of the waterborn disease outbreaks and for 49% of the cases from private water systems, brought about by people drinking untreated ground water over the years 1946 to 1970. Furthermore, these private water systems produced 253 outbreaks or 71% of the total outbreaks in the United States during this time period.

2. Working Hypothesis

The above studies together with those presented in the review of literature (Chapter 2) pointed to the possibility of ground water pollution by landfill leachate microorganisms. In order to demonstrate how such pollution could occur it was necessary first to determine the evidence for microbial survival both in newly deposited landfills and those several years old, and to show how microbes can pass down vertically to the ground water below. Furthermore, it

would be necessary to show that microorganisms could not only survive ground water conditions, but could besides moving vertically could also move laterally, making it possible to contaminate nearby drinking water sources. This projected pathway of microbial travel from solid waste to ground water is presented in Figure 1. Also, it was important to note that municipal waste contained infectious microorganisms. If these microorganisms could survive for long periods of time in the soil or landfill, then they might be resistant enough to be present in the leachate when it left the landfill and would be able to pollute the surrounding environment. Consequently, the working hypothesis on which this present study was based was that the pathogenic microorganisms present in solid waste can survive landfill conditions, thus making it possible for them to drain out with the leachate and contaminate the soil and water surrounding the landfill.

B. OBJECTIVES

To carry out these objectives the following experimental approach was taken:

Phase I - The Development of Methods

Phase II - Studies on Simulated Landfills

(Lysimeters)

Phase III - Field Studies on Full-scale Landfills

<u>Municipal Solid Waste</u>	<u>Infectious Wastes</u>
Household Refuse Rubbish Garbage Yard Wastes	Household Wastes Baby's Diapers Dead Animals Animal Feces
Commerical Wastes Food Wastes Paper	Hospital Wastes Operation Room Wastes Bandages Baby's Diapers
Industrial Wastes Rubbish Inert Chemicals	Sewage Sludge Slaughter House Wastes Veterinary Wastes

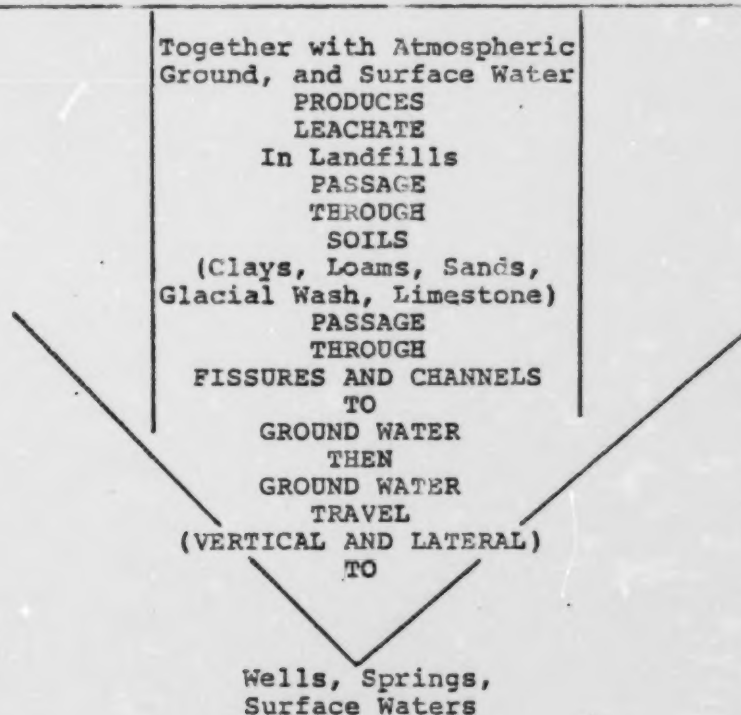


FIGURE 1. POSSIBLE PATHWAYS OF MICROORGANISMS WASHED OUT FROM LANDFILLS BY LAND AND SURFACE WATER.

In Phase I problems were studied and new methods were developed. Problems such as the freezing of leachate samples, leachate toxicity, most-probable-number test non-specificity, and bacterial stress due to leachate toxicity were examined. The development of methods involved the improvement of a Salmonella enrichment medium, the concentration of leachate for the enumeration of Mycobacterium, and a comparison of eight streptococcal enumeration procedures, the adaption of clostridial enumeration procedures, and the development of methane utilization and methane producing procedures. In Phase II, six lysimeters were constructed and placed at the University of Cincinnati to test the survival of pathogens in various combinations of hospital waste, sewage sludge, and municipal solid waste. After two to three years, these six lysimeters were opened and the solid waste within were examined for surviving fecal indicator bacteria and pathogens. Finally in Phase III the commercial landfill leachate from the commercial landfill was used for the streptococcal, salmonella, mycobacterial and clostridial isolations. Also, closure studies on the Boone County landfill gave an opportunity to check similar type studies made previously on lysimeters. Finally, methane utilizing and methane producing bacteria assays were made on leachate, and solid waste from this same landfill.

CHAPTER II

LITERATURE REVIEW

A. INFECTIOUS MICROORGANISMS IN LANDFILL WASTE

1. Municipal Solid Waste

Municipal solid waste consists of household and commercial items, The classification of these items, their individual proportions in the waste, and their percentage of moisture for 1968 is presented in Table 1. To analyze this waste for bacteria most investigators have used viability counts reported as colony forming units, or CFU, per gram of the waste (CFU/g); fecal indicator tests; and actual microbial identifications i.e. speciation . Standard plate counts have yielded values of 10^7 to 10^8 CFU/g and the fecal indicator levels (reported as MPN/g) ranged from 10^3 to 10^5 for the fecal coliforms, and 10^3 to 10^5 for the fecal streptococci (Carpenter and Setter, 1940; Dobson, 1964; Cromwell, 1965; Peterson and Stutzenberger, 1969).

Examination of the municipal solid waste has included the identification of microorganisms. For example, Cromwell (1965) identified Pseudomonas sp. as the most frequent isolate from solid waste and identified several species of this genus. Other identified microorganisms include the following: Achromobacter delicatulum, A. isophagus, Bacillus sp. Flavobacterium aquatile, Clostridium sp. Neurospora

TABLE 1. The 1968 Municipal Refuse Characteristics^a

Category	Discarded Refuse ^b Percent	Moisture ^c when Discarded Percent
Glass	8.1	2.0
Metal	8.1	2.0
Paper	35.1	8.0
Plastics	1.1	2.0
Leather, Rubber	1.4	2.0
Textiles	1.9	10.0
Wood	2.4	15.0
Food Wastes	19.5	70.0
Yard Wastes	20.7	50.0
Miscellaneous	1.7	2.0

a - From Niessen and Chansky (1970)

b - Percentages reported for semi-seasonal states, e.g. Alabama. Not listed here were the unseasonal states such as Florida and the seasonal states such as Massachusetts.

c - Bacteria require moisture for survival. Pathogens and fecal indicator bacteria would most likely survive on the waste containing the higher moisture content.

crassa, Rhizopus nigricans, Chaetomium sp., Trichoderma sp., Aspergillus niger, Aspergillus flavus, Ceratocystis sp., Fusarium sp., and Penicillium sp. Using the membrane filter she found Escherichia coli and Streptococcus faecalis concentrations of 5,000 and 2,880 CFU/g (dry weight), respectively. Moreover, Peterson and Klee (1971) isolated Salmonella from the solid waste/sludge mixture, municipal solid waste, and from incinerator residue and quench water. Commercial waste is also included in municipal waste, e.g. slaughter house waste (Solid Waste Disposal Act, 1965). One recent paper by McKinley et al. (1980) described the search for Salmonella in nine swine slaughterhouse plants, and nineteen swine production units from six states. These latter units represented 64% of the United States swine production. These investigators isolated Salmonellae from seven of the nine slaughterhouse plants and three of the nineteen production units to give a total of 52 isolates. This presence of Salmonella in the meat scraps suggests that other fecal pathogens could also be present in the waste.

The first viral studies were reported by Peterson (1971, 1975). In this work she developed methods, and analyzed municipal solid waste for viruses. The two procedures developed for extracting and concentrating viruses from solid waste and leachate were ultracentrifugation and resin membrane adsorption. The ultracentrifugation averaged

a recovery rate of 36% for polio virus and 66% for coxsackievirus B2. However, the resin membrane filtration method was ineffective as 90% of the poliovirus passed through the membrane. She reasoned that the cause of the viral elution was due to the competition of the soluble organic substances in the solid waste for the absorption sites on the membrane. Then she applied these procedures to field studies. First she assayed municipal waste prior to its deposition in a landfill. From this waste she isolated type 3 poliovirus from four solid waste samples and one disposable diaper using the ultra centrifugation procedure, and she isolated poliovirus types 1, 2 and 3 from one solid waste sample using the resin membrane absorption method. The poliovirus type 3 strains showed a prevalence of d^+T^+ and $d^+T^\pm$ strains suggesting perhaps the presence of a wild strain from the community or a genetic reverted marker from a vaccine strain. Second, she examined eight tons of municipal waste and found that 0.6 to 2.4% of the waste contained soiled disposable diapers. Thirty-three percent of the diapers (84) contained fecal matter. Each pound (wet weight) of feces soiled diapers contained an average of 60 g of feces. She examined the feces for polio-type viruses. Her isolates showed the presence of d^+T^+ and $d^+T^\pm$ strains as found above. She estimated that the enteric virus density in the solid waste was about 64 PFU/200 g.

Feces from baby's diapers contribute a variety of microorganisms to landfills, some of which might be infectious to humans. Using a computer search, Scherago (1972) found that a large number of pathogenic viruses, bacteria, and parasites could be brought to the landfill in these diapers. His results are listed in Table 2. The computer search revealed no reports of pathogens that might occur in sanitary landfills and leachates from them. Also related to this only two reports were found that told of the presence of pathogens in solid waste.

2. Hospital Waste

Hospital waste contains a variety of materials such as garbage, lunchroom wastes, operating room wastes, baby's diapers, dressings and many others, some of which may contain pathogenic microorganisms stemming from patients infections. These wastes were not usually autoclaved, and so they contained Staphylococcus sp., Streptococcus sp., Mycobacterium tuberculosis, Klebsiella sp., and Salmonella sp., which may eventually be discarded in landfills. A study on wastes from a West Virginia hospital and a nearby medical complex was reported by Wallace et al., (1972). They found that the solid waste generated per person per day was 3.6 lbs., which was within the range (3.1 to 5.6 lb) of a similar Los Angeles hospital study referenced in their

TABLE 2. Diseases Associated with Human Wastes and
the Causative Organisms (from Scherago, 1972)

1. Bacterial Infections:

a. Typhoid fever - Salmonella typhi

b. Paratyphoid fever - Salmonella paratyphi A

- Salmonella paratyphi B

- Salmonella paratyphi C

- Salmonella sendai

- Salmonella typhimurium

c. Bacillary dysentery (Shigellosis)

Shigella dysenteriae

Shigella flexneri

Shigella boydii

Shignella sonnei

Escherichia coli, certain serotypes produce a
severe type of epidemic diarrhea in infants.

d. Salmonellosis - Acute gastroenteritis

Salmonella types (out of 1200) most common in
the period 1963-1968 in U.S.A. were S. typhimurium,
S. heidelberg, S. newport, S. infantis,
S. enteritidis, S. derby, S. st. paul.

Other less common species of enteric bacteria:

Edwardsiella tarda, Erwinia carotovora, Serratia
marcescens, Neisseria lactamicus.

TABLE 2. (Cont.)

-
-
- e. Pseudomoniasis - Pseudomonas aeruginosa
 - f. Cholera - Vibrio cholerae
 - 2. Viral Infections
 - a. Picornaviruses of human origin
 - Enterovirus group
 - Poliomyelitis)
 - Coxsackie A) Numerous
 - Coxsackie B) serotypes
 - Echoviruses)
 - b. Peoviruses
 - c. Infectious hepatitis
 - 3. Fungus Infections
 - a. Candidiasis - Candida albicans
 - 4. Animal Parasites
 - a. Protozoa
 - (1) Amebiasis
 - Entamoeba histolytica
 - Entamoeba coli
 - Endolimax nana
 - Iodamoeba bütschlii
 - (2) Balantidiasis
 - Balantidium coli

TABLE 2. (Cont.)

(3) Giardiasis

Giardia lamblia

b. Nematodes

Ascaris lumbricoides

Trichuris trichiura

Enterobius vermicularis

Ancylostoma duodenale

Strongyloides stercoralis

c. Cestodes

Tenia saginata

Tenia solium

Hymenolepis nana

paper. In the West Virginia study, the investigators found that only 25 to 30% of the total waste could be considered infectious or potentially dangerous. They concluded that high numbers of pathogenic organisms were present in the waste. These included isolates of: Staphylococcus aureus predominant pathogen detected; Streptococcus sp., (each coccus type was 5 to 10% of the observed population); high numbers of coliforms from certain units; and 80 to 90% Bacillus sp. Finally, they stressed the importance of considering all hospital trash to be contaminated when the general trash was mixed with the infectious waste.

Hospital waste has increased during the past several years from the 3.6 lbs. per capita, as determined by Wallace et al., in 1972, to 10 to 40 lbs. per patient per day, as reported by Block and Netherton in 1977. The wide range was believed due to the variable quantity of disposable items in use in different hospitals. Although incineration of hospital wastes was noted to have considerable potential, and theoretically could destroy all combustible wastes--thus rendering them noninfectious in practice, however, incineration is ineffective (Block and Netherton (1977)). Thus, most hospital wastes are disposed of in landfills. Block and Netherton (1977) urged that hospitals sterilize such wastes before land-filling to avoid the possibility of the spread of infection and disease due to the presence of

viable microbial pathogens. The effect of "pathological" waste materials are minimized by physical separation of the waste in the hospital and subsequent treatment (usually incineration) before actual disposal. A myriad of constituents possibly containing disease-producing microbes are found in such "pathological" hospital waste, and include dressings, contaminated disposable items, surgical drapes and human tissue from surgery, bacteriological cultures, urine, blood, and fecal samples from the laboratory and pathology department, animal remains from medical experimentation and miscellaneous infectious material as gauze, bandages, swabs, plaster casts, sputum cups, tissues soaked with nose or throat secretions, and wound drainage from the hospital wards. Since only 25 to 30% of the total waste generated in a hospital can be considered dangerous, the total bulk of the waste need not be treated as being contaminated. However, another viewpoint is becoming popular, where a difference is noted between "pathological" waste and simply "infectious" wastes. According to this viewpoint, pathological materials are those from surgery, a laboratory, whereas infectious waste is everything else--everything on the hospital floor that comes in contact with the patient (Block and Netherton, 1977). The Esco/Greenleaf (1972) report concluded that "...everything from a hospital floor must be considered to be contaminated.."

Virus survival studies done by Burchinol and Wallace (1971) and Wallace et al. (1972) showed that paper and cotton fabrics held active viruses for long periods of time, generally from 5 to 8 days. Virus titers were found to decrease in most cases at a steady rate with increasing time. Thus, viruses may lose their viability upon inoculation.

The increased use in hospitals of disposable items has (as noted previously), partly resulted in the increase in the quantity of solid waste generated by hospitals. Disposables are attractive for hospital use because of the decreased danger of cross-infection and supposedly greater economy in using them. Disposables include a myraid of items that are combinations of materials such as paper, plastic, (such as polyoefins, polystyrene, and polyvinyl chloride), found through-out hospital wards, nursing stations, laboratories, surgery, and food service. They include gowns, sheets (bed), pillow cases, towels, wash rags, handkerchiefs, bed pans, all kind of oral, urinal and anal drainage systems, drapes, operating gowns, gloves, sponges, tongue blades, all kinds of sterile irrigation trays, baby bottles, bibs, and diapers. The increased use of these disposable items has brought their total cost up from 20 million dollars in 1955 to 300 million in 1971 to a projected cost of \$900 million in 1978. Hospital solid waste has a

considerably higher plastic content than the 2 to 3% reported in municipal solid waste. Besides increasing the volume of the solid waste, plastic disposables are difficult to degrade "cleanly", even by incineration (Block and Netherton, 1977). The incineration of some items, especially plastics may produce particulates, toxic combustion products, and even leave some items unburned.

In a recent report, Sparks (1981) noted that before the 1960's the amount of solid waste generated per patient per day was 7 lbs., but had increased in 1981 to 15 to 20 lbs. and, in special cases, up to 30 lbs. Hospital wastes to be discarded in landfills included : trash - paper, cardboard, wood; garbage - animal and vegetable material from institutional food preparation; food service waste - garbage, plastics, paper, waste containers; pathological waste - organs, laboratory animals, contaminated waste - all items in contact with the patient and therefore may be infectious; other - construction scraps, sweepings, needles, and radio-active waste, In addition to the above, Block and Netherton (1977) noted that veterinary clinics send their dead animals and medical clinics send their waste material to landfills. These wastes could contain animal and human pathogens.

Three ways to dispose of these hospital wastes include: grind it up and discharge it to a sewage treatment

plant, take it to an incinerator site, and to bury it in a landfill. However, there are certain disadvantages with discarding the waste in landfills. For example, at the landfill the waste will come in contact with refuse workers and scavengers. Also, rain and run-off will leach the material and this may affect the populace. Finally, Block and Netherton (1977) concluded that communities placed wastes in the landfill to destroy or get rid of the pathogens and prevent transmission of disease. Consequently, community health standards may well demand that the waste be rendered harmless before it leaves the hospital.

Block and Netherton's conclusions were born out by field studies. Hospitals with tuberculosis patients present a special problem in that the sewage treatment do not always destroy Mycobacterium tuberculosis in septic tanks, sedimentation, and biological filters (trickling filters) without additional treatment. Jensen (1954) reported on the examination of sewage from five Denmark towns in which tuberculosis institutions were situated, and from a large institution for the care of the feeble-minded which had a tuberculosis ward. All influents to the sewage-treatment plants contained tubercle bacilli. Preliminary sedimentation of the town plant influents did not remove the tubercle bacilli. Also, humus tank effluents in two towns using trickling filters followed by humus tanks contained tubercle bacilli.

Although activated sludge effluent from one of the towns contained no tubercle bacilli, they were present in effluent from an activated sludge plant used to treat tuberculosis sanitarium waste. Jensen concluded that the entry of even a small quantity of untreated sewage containing tubercle bacilli from a tuberculosis institution to the sewage system of a community, may infect the town's sewage and sludge. Studies were thus also conducted to determine the longevity of tubercle bacilli in sewage sludge placed in drying beds. He found that it took 11-1/2 to 15 months before the tubercle bacilli could no longer be detected.

Finally, Norris and Young (1978), from the Maryland Department of Health and Mental Hygiene, received reports of human tissue, bandages, and other inappropriate, unaesthetic materials visible in landfills. They recommended onsite incineration or sterilization of such hazardous waste. They indicated that the number of pathogens considered to be hazardous in waste is increasing as microorganisms once commonly thought to be nonpathogenic such as pseudomonads, flavobacteria, chromobacteria, and candida, have been shown to be involved in the disease process.

3. Sewage Sludge

Sewage sludge in landfills possibly contributes a greater variety of pathogens than the hospital wastes, and

certainly the concentration is greater. This waste comes from all parts of the city and includes sewage from healthy and hospitalized individuals. Also included are the infectious agents currently inflicting the population at the time the sample are taken as poliovirus, hepatitis virus, adenovirus, and coxsackie viruses. Additional microorganisms found in sewage sludge are: Salmonella typhi, Vibrio cholerae, Shigella, Clostridium perfringens, Mycobacterium tuberculosis, Klebsiella, Allerscheria boydii, viruses, and parasites (Heukelkian and Albanese, 1956; Kabler, 1959; Foster and Engelbrecht, 1973; and Love et al., 1975). The pathogenic microorganisms in sewage sludge are of great variety and appear in high concentrations, making this addition to landfills a hazard. The following research studies provide additional information, although the waste water effluent studied was more dilute than the sewage sludge. Guentzel (1975) found this waste water effluent to have a standard plate count of 10^8 /100 ml and to contain the following microbial concentrations (CFU/100 ml): fecal streptococci, 10^3 ; Clostridium perfringens, 10^3 ; fluorescent pseudomonads, 10^5 ; Klebsiella, 10^4 ; Staphylococcus aureus, 10^3 ; and mycobacteria, 10^5 . In addition, partial list of microorganisms found in sewage by Sagik et al., (1980) is presented in Table 3.

TABLE 3. Microorganisms Reported Present in Sewage

Identified Isolates From		
Digested Sludges Microorganisms ^a	Range CFU/g	Sewage Sludges, Microorganisms ^b
Total Coliforms	10 ⁶ -10 ⁷	Viruses:
Fecal Coliforms	10 ⁵ -10 ⁶	Poliovirus
Fecal Streptococci	10 ⁴ -10 ⁵	Coxsackievirus
<u>Staphylococcus</u> sp.	10 ⁴ -10 ⁶	Echovirus
<u>Clostridium perfringens</u>	10 ⁷	Reovirus
<u>Mycobacterium</u> sp.	10 ⁷	Adenovirus
<u>Salmonella</u> sp.	≤ 2	Hepatitis virus
<u>Citrobacter freundii</u>	10 ⁵ -10 ⁶	Parasites:
<u>Enterobacter aerogenes</u>	10 ⁶	<u>Giardia lamblia</u>
<u>Escherichia coli</u>	10 ⁵ -10 ⁶	Liver and Intestinal Flukes
<u>Serratia marcescens</u>	10 ⁵	<u>Entamoeba histolytica</u>
<u>Yersinia interocolitica</u>	10 ⁵	<u>Ascaris lumbricoides</u>
<u>Proteus morganii</u>	10 ⁵	<u>Ancylostoma duodenale</u>
<u>Acinetobacter</u> sp.	10 ⁵	<u>Necator americanus</u>
<u>Aeromonas hydrophila</u>	10 ⁶ -10 ⁷	<u>Taenia saginata</u>
<u>Klebsiella pneumoniae</u>	10 ⁵ -10 ⁶	<u>Trichuris trichiura</u>

a From Sagik, Duboise & Sorber (1980). Recovered on MacConkey or xylose lysine desocholate agar and identified using API strips. One hundred colonies per plate were identified.

b From Love et al. (1975), and Burge and Marsh (1978).

B. CHEMICAL CONDITIONS WITHIN THE LANDFILL

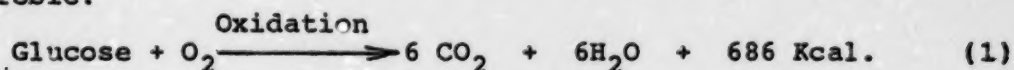
1. Overview

As noted in the discussion of waste contents, the microorganisms from the municipal solid waste placed in landfills are among the most resistant and the most pathogenic forms known to mankind. However, their survival within landfills had not been demonstrated until recently, although studies of microbial survival in soil, and sewage sludge have shown microbial viability of a year or so (Jensen, 1954, Hutchenson, 1974). Undoubtly the survival of these microbes depend upon the particular internal environment of the landfill. A part of this environment may be described by chemical conditions occurring within the landfill.

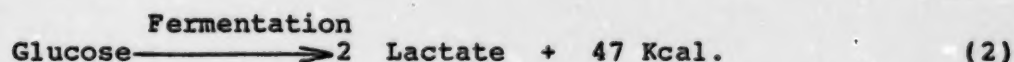
The landfill chemical reactions are similar to those conditions in nature that aerobically and anaerobically decompose carboydrates, protein and fats to produce acids, water, CO_2 and CH_4 . Examples of such reactions would be the oxidation of organic material, and the anaerobic digestion of sewage sludge, pond or lake debris, anaerobic lagoons, or a waste stabilization pond. The initial metabolic reactions are aerobic until the microorganisms use up all of the oxygen. Then the anaerobic digestive reactions proceed. The aerobic hydrolysis of carbohydrates release large amount of energy, particularly when compared to the smaller amounts

produced by the anaerobic reactions. The oxidation and fermentation of glucose is presented in the following reactions (Lehninger, 1975):

Aerobic:



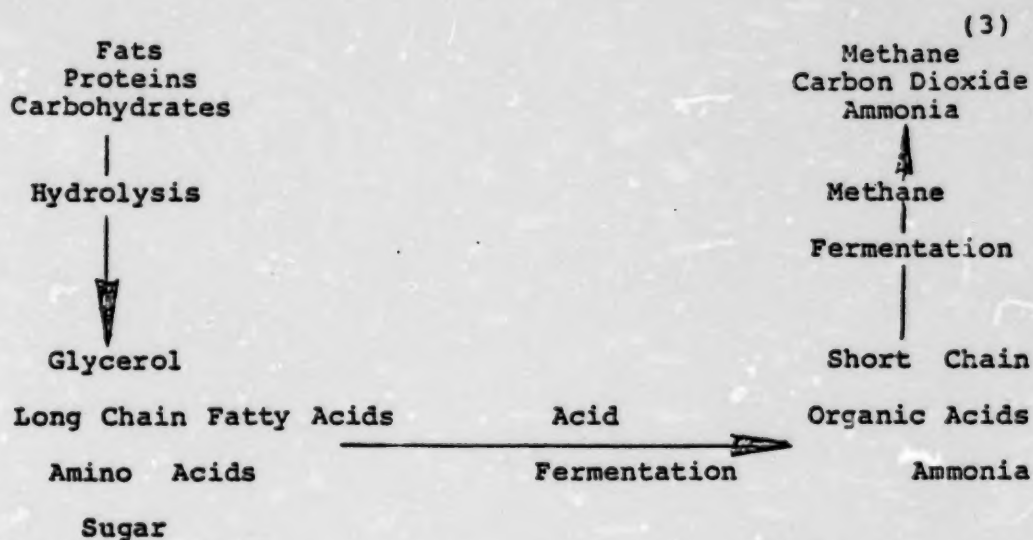
Anaerobic:



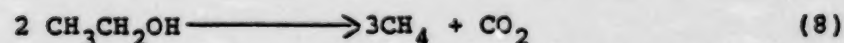
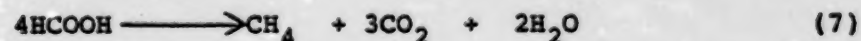
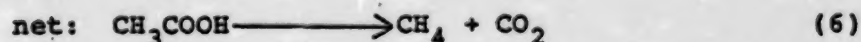
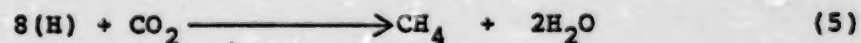
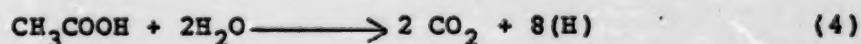
These, of course, are only a portion of the substrates and the products. See Table 4 for a more extensive list of the products. The main point to be made here is that the aerobic reactions produce a large amount of heat which is responsible for the high temperatures found in solid waste collection. These temperatures may rise from 100 F to 150 F and have been regarded by some investigators as deleterious to the pathogens in the waste (Engelbrecht et al., 1974). Kugelman (1981) and Jeris and Hughes (1980) extended these reactions to explain anaerobic sludge digestion which occurs by extra-cellular enzyme digestion derived from several groups of microorganisms which produce acids, CO_2 , H_2 , etc. as seen in the following reactions:

TABLE 4. Microbial Products Found in Leachate
(from Stanforth et al., 1979, and Lin, 1965)

Chemical Groups	Grouped according to product sources or chemical similarity
<u>Products Derived From:</u> 1. Carbohydrate Stabilization	Short Chain Alcohols Alkanes Ketones Esters Chloroform Benzene Toluene Xylene
2. Proteins Stabilization	Amino Acids NH_3
3. Lipids Stabilization	n-Valeric Acid iso-Valeric Acid n-Butyric Acid iso-Butyric Acid Propionic Acid Acetic Acid Caproic Acid
<u>Chemical Similarity</u> Ions	Ca^{++} Fe^{++} Zn^{++} HCO_3^- SO_4^- Cl^- H_2PO_4^- H_2^{++} Mg
Gases generated	H_2 CO_2 CH_4



The acids, particularly acetic acid, when all the oxygen has gone, will be metabolized by the Methanobacterium, Methanosarcinae, and the Methanococcus to CO_2 and CH_4 , Alexander (1977). The equations for these reactions showing the production of methane are:



2. Leachate Compositon

Leachate is produced by water perculating down through

the landfill. This water comes from precipitation, surface runoff, ground water from adjacent areas, a rise in the ground water level at the site following the deposition of refuse, moisture from the refuse itself, and possibly water resulting from the aerobic decomposition of organic material. Following this, the solid waste begins to decompose and produces gases and liquids by aerobic decomposition and by anaerobic decomposition.

A number of factors affect these reactions. If the solid waste in the landfill is relatively dry and only contains 20 to 40% moisture, then stabilization, i.e., the decomposition reactions with the productions of acids, CO_2 and CH_4 in minimal amounts, will continue slowly. The optimum conditions for biological activity is 40 to 60% moisture. In the presence of oxygen, aerobic decomposition occurs and when this gas is used up anaerobic conditions begin. Temperatures affect these reactions. Temperatures ranging from 95 to 100 F in the mesophilic interval and above 110 F for the thermophilic range. Degradation is faster under the thermophilic conditions. Finally, the pH has an effect on the methane production. The methane production occurs over a range of 6.6 to 7.6. This methane reaction, a secondary reaction occurring after the initial decomposition of landfill waste, utilizes these products to produce methane. When the acid formation is greater than

the methane formation, then the methane levels decrease, the CO_2 increases and the pH remains low (Chen and Bowerman, 1974).

The ionic strength may affect the solvent activity of the leachate by, one, increasing the solubility because of the lowered activity coefficients, second, the transfer of ions-bound with a predominant ion in solution, and third, promoting coagulation by decreasing the double layer size around the colloidal particles (Stanforth et al., 1979). This group also reported charge balance calculations for the Boone County landfill using milliequivalents per liter for the positive ions: Na^+ , 32.6; K^+ , 22.0; NH_4^+ , 17.7; Ca^{++} , 116.0; Mg^{++} , 43.6; Zn^{++} , 1.6; Fe^{++} , 26.1; Total positive charge, 259.9; negative charge: volatile acids (using pK), 55.1; HCO_3^- , 1.8; $\text{SO}_4^{=}$, 19.0; Cl^- , 59.0; NO_3^- , 0.7; H_2PO_4^- , 0.0; total negative charge, 135.6; apparent net charge, 124.3⁺. As the number of positive charges exceed the negative charges, it is obvious that there are many negative ions not accounted for in the above list (Stanforth et al, 1979). The importance of these ions for bacteria is not known. However, these metal ions may affect bacterial survival or their chemical activities (Block et al., 1977; and Davis, et al., 1980).

The reactions describing the anaerobic digestion of sewage sludge are similar to the landfill digestion process. Extensive studies were made by Chien and DeWalle (1975,

1977) on landfill leachate chemistry by standardizing the leachate chemical analytical procedures. They noted the sequence of leachate products appearing within the landfill with time. Because of the fine work of these investigators, comparative data was available from several landfills over a period of several years, so that Stanforth et al., (1979) were able to set forth a theoretical degradation curve for a theoretical landfill. Stanforth et al. (1979) selected the parameters most important to bring about landfill stabilization, i.e., those parameters which changed with the aging process, such as pH, oxidation-reduction potential, organic solvents, ionic strength, and the gaseous composition. The metabolic stages present in landfills was described by Stanforth et al. (1979). They showed that the volatile acids (acetic acid) increased in concentration with an increase in time, and then decreased as they were used up in the reaction, probably for methane production. These reactions suggested that the solids were liquified with time. The pH decreased with an increase in acid production, and increased to neutral as the acid was used up. As the pH was neutralized, the methane production increased significantly. Thus Stanforth et al., (1979) were able to perceive of two main phases of lysimeter and landfill metabolic activities as an aerobic phase and an anaerobic phase, the latter being completed in two stages of several years duration. The

significance of three landfill degradation stages as described by Stanforth et al., (1979) are summerized below:

Aerobic Stage -

The aerobic degradation reactions used up most of the oxygen and produced CO_2 . BOD was initially high. A large amount of heat was produced. This raised the waste temperature above the ambient temperature. See equation 1 above. The acid produced helped to dissolve more waste material. Most of the very soluble material was leached at this stage.

First Anaerobic Stage -

The waste degradation by the facultative anaerobes continued to use up all of the oxygen, and the resultant anaerobic reactions began. The highest concentration of CO_2 (60%) was produced, and the methane production was initiated to produce minimal amounts up to 15%. Maximum acid production occured (about 18,000 ppm acetic acid). The pH lowered to 4 to 5, which maximized the conductivity. Metal ions were produced. High volatile acids contributed high COD.

Second Anaerobic Stage -

The utilization of the acids raised the pH, and lowered the conductivity. The CO_2 was used to make some of the CH_4 (50% maximum) which decreased its

percentage to a steady 20%. The ORP lowered still further. The ammonium ion was produced. The volatile acids were converted to CH_4 and CO_2 . Methane production required a pH of 6.6 to 7.3. With this higher pH there were less extractions of inorganic ions, and conductivity levels decreased.

A further understanding of the reaction sequence within landfills may be seen with the results of Chian and DeWalle (1977). Their work indicated that the carbohydrates and volatile acids (50%) were attacked within the first year, while the rest of the organic carbon (70% of the TOC) and the proteins and amino acids (20%) reached the highest level in the second year. The organic carbons continued to be released to the 14th year while the proteins and amino acids were released in small amounts, indefinitely. In addition, they related the COD, BOD, and TOC to the ages of the landfill. These relationships were:

1. < 5 years, the COD = 10,000 mg/l
2. 5 to 10 years, the COD = 500 to 10,000 mg/l
3. >10 years, the COD = 500 mg/l

C. MICROBIAL SURVIVAL IN LEACHATE

1. Purpose of Study and Methods

Hanks (1967) in his review of Solid Waste/Disease Relationships stated that the morbidity of a number of human

fecal-borne diseases in the United States was high and suggested that considerable environmental fecal contamination occurred. But which transmission route was important was essentially unknown as was also the influence of insanitary foci on the overall incidence of these diseases. Therefore, there may be significant fecal contamination present in the environment, but how it transmits diseases to humans is unknown. Consequently, Hank's conclusions signified the importance of the proper disposal of human waste because of the fecal pathogens. The proper disposal of human solid waste from municipal sources was considered to be sanitary landfills. It was during this period and the following ten years that investigations evaluating the survival of microorganisms in landfills were begun.

Analyses were made on leachate samples to determine the presence of pathogens or more often the presence of fecal indicator bacteria in landfills, which survive these conditions and which may leave landfills in the leachate and enter the ground water or surface waters. To this end the initial investigators used full scale landfills for testing the microbial indicator survival. Later studies determined the number of bacteria and the various groups of microorganisms present. After Qasim (1965) used lysimeters, many other investigators constructed similar units for studying various types and combinations of wastes. For example,

nineteen lysimeters were built at the Center Hill Laboratory (EPA) in Cincinnati, Ohio in 1974, 1975. These units are still being assayed at the present time to determine the effect of co-disposal of industrial wastes on municipal solid waste under simulated landfill conditions (Jackson and Streng, 1976; Streng, 1976; Walsh et al., 1979, 1981). However, the microbial studies were not always conclusive for several reasons. For example, the lysimeter incubation temperature varied, a variety of wastes were used, there was leachate toxicity, particularly in old lysimeters, differences in laboratory methods, and finally lysimeters or landfills were used. The results did not indicate that fecal material in the form of leachate leaking from landfills would be a problem as the fecal indicator levels usually decreased significantly to very low numbers or to zero within a year, especially within the lysimeters, or the simulated landfills (Engelbrecht et al., 1974, Cooper et al., 1974; and Ware (1980).

Originally the landfills themselves, were sampled. One of the early methods used to take landfill samples for evaluation (Carpenter and Setter, 1940) was to dig a hole into the waste and allow leachate to accumulate in the hole overnight. This liquid, produced by the presence of rain water and by its extraction of the soluble waste material, is called landfill leachate. This leachate was also produced by the lysimeters (Qasim, 1965).

These lysimeters, used by Qasim (1965) and others, consisted of large tanks filled with garbage and other municipal waste, were covered with soil or on other occasions left open to the air. They were placed at ground level or were buried in the soil, and were watered periodically to simulate rainfall for a particular part of the country. No doubt the remoteness of the landfills from the laboratory prompted the use of lysimeters. Once in use, the lysimeters offered not only a way to define the environmental conditions, but a way to vary the waste content and learn about the stages of decomposition over a span of several years. The chemical stages have already been described (Pohland, 1975; and Stanforth et al., 1979). On the other hand, the microbial stages have not been studied extensively.

The laboratory methods used in the evaluation of landfill pollution by leachate include procedures taken from Standard Methods beginning with Carpenter and Setter, (1940), namely BOD, alkalinity, acidity, pH, later on COD, conductivity, total nitrogen, and methods for determining fecal indicator bacteria (total coliforms, fecal coliforms, and the fecal streptococci), such as membrane filtration and the most probable number (MPN) tests (Engelbrecht et al., 1974, Cooper et al., 1974, and Glotzbecker, 1974). Later Chian and DeWalle (1978) and Peterson (1973) developed procedures especially for use in analyzing solid waste and leachate.

2. Early Studies on Microbial Survival

The early investigators of solid waste pollution assayed leachate and waste from full-scale landfills. One such investigation of solid waste pollution was done by Carpenter and Setter in 1940. They examined five of the fullscale landfills near New York City, three of which were less than two years old and the fourth was two to four years old and the fifth was 30 to 35 years old. The waste from one of the newer landfills contained total coliforms and fecal coliforms of 7.4×10^5 MPN/g and 3.3×10^5 MPN/g, respectively. In addition, they took leachate samples from bored holes at various depths in the landfill waste. The leachate which collected in the hole after a 12 hour period was taken as the sample. These samples had an average pH of 6.9 with a range of 5.7 to 8.4. The fecal coliform count was 2.45×10^3 /ml. The total alkalinity and acidity (ppm) were 2,867 and 1,089, respectively. The dissolved oxygen was 1.2 ppm. Dobson (1964) also studied a municipal sanitary landfill that was two or more years old in Fairmont, West Virginia. She examined the initial high temperatures that most solid waste samples produce and found that aerobic conditions were necessary for the production of maximum temperatures in the newly deposited waste. Dobson believed that this high temperature over a period of time, which

indicated continued aerobic conditions, decreased when anaerobic conditions were present. Microbial counts increased under aerobic conditions, and then decreased under anaerobic conditions. Oxygen depletion was used to estimate the respiration rates in the determination of microbial activity. Dobson found that 60% moisture over a range of 40 and 80% gave the highest microbial activity. In addition, she dug several holes in landfills and found that over all, the pH ranged from 5.1 to 7.3 and the moisture varied from 28 to 74%. However, in household refuse the pH varied from 4.9 to 5.8 and the moisture varied from 42 to 59%. Also, Dobson found that mixed refuse had a high buffering capacity; but newspapers, also present in the waste, did not contain this property.

A year later, Cromwell (1965) studied refuse from the same landfill, which was at this time three or more years old. The decomposing waste contained large numbers of Bacillus sp. and several species were identified. Also, the following species were identified : Streptomyces sp., Clostridium sp., Lactobacillus sp., Propionibacterium sp., Sarcina sp., Aspergillus sp., Penicillium sp., Trichoderma sp., Epicoccum sp., Sphaeronema sp., Fusarium sp., Zygorhynchus sp., Neurospora crassa and Rhizopus nigricans. Finally, the fecal indicators present in the decomposing waste had levels of: Escherichia

coli, 100 CFU/g, and Streptococcus faecalis, 60 CFU/g of waste (average of 3 samples using the membrane filter).

In addition, Cook (1966) and Cook et al., (1967) continued the study of the Fairmont sanitary landfill in Fairmont, West Virginia. This time the waste was more than four years old. He examined both the external leachate samples and the leachate samples taken from within the landfill (internal). Those microbes, found both in the external and internal leachate samples, were: Pseudomonas sp.; Bacillus sp.; Brevibacterium sp.; Micrococcus roseus, Lactobacillus sp.; Micromonospora sp.; Streptococcus sp.; coliforms, Clostridium pictinovorum and Streptomyces sp. He found many species of algae. Those bacteria found only in the external leachate samples included the following: Achromobacter sp.; Corynebacterium hoagii, Flavobacterium sp.; Kurthia bessonii, Rhodopseudomonas sp.; Alternaria sp.; Aspergillus sp.; Chalaropsis sp.; Cladosporium sp., Cladotrichum sp.; Fusarium sp.; Gliocladium sp.; Humicola sp.; Margarinomyces sp.; Neurospora sp.; Paecilomyces sp.; Penicillium sp.; Pullularia sp.; Spicaria sp.; Trichoderma sp., and Rhodotorula sp. Those found only in the internal leachate were certain species of: Brevibacterium; Pseudomonas; Arthrobacter; Microomospora; and Nocardia. Cook examined both types of leachates for the presence of fecal indicator bacteria. He found coliform levels of roughly 300

to 5,000/ml and enterococcus levels of 90 to 1,300 per ml in the external leachate seepage from the landfill. The coliform levels in the interior leachate samples were 160/ml to 350/ml and the enterococci were present in levels of 5 to 77/ml in the samples. He identified the coliforms as Escherichia coli and Escherichia freundii. No Enterobacter sp. were found. The enterococci were identified as Streptococcus faecalis. The 20 to 35 mg/L of total iron in the external leachate probably came from the buried metals. Thus, this landfill was old and therefore had passed its initial period of decomposition. The pH was always neutral or slightly alkaline. Also, there were nitrifying bacteria, which were largely responsible for the conversion of ammonia to nitrate. Furthermore, he found some bacteria which would change the nitrates to ammonia.

3. The Initial Use of Lysimeters for Landfill Studies

Qasim (1965) used lysimeters instead of full-scale landfills. His lysimeters, contained mixed municipal refuse placed in three concrete cylinders that were 3 ft in diameter and 4, 8 and 12 ft in height, which were labeled A, B, and C, respectively. On top of each cylinder he placed 12 inches of soil. The maximum initial rise in temperature for the A, B, and C lysimeters were 88.7, 92.0, and 141.5 F,

respectively. The initial total coliform levels in the cylinders were 8.5×10^6 , 10.75×10^6 , and 1.3×10^6 MPN/100 ml of leachate in the A, B, and C lysimeters, respectively. The study lasted for 163 days. The MPN levels decreased for cylinders A and B in 163 days and decreased for C in 121 days, by 99.6%, 99.8%, and 97.5%, respectively. Although it was difficult to interpret the MacConkey agar plate results, it appeared that the Escherichia coli died out in about ten weeks. Thus, the decrease in microbial levels were related to the decrease in pH as the latter occurred just prior to the bacterial population drop.

The parameters which appeared to affect the leachate characteristics were the landfill moisture content, temperature, and refuse composition (Pavoni et al., 1975). Earlier, Eliassen (1942) reported that the optimum moisture content (50 to 70%) for recently landfilled material served to increase biological activity and to increase the rate of waste degeneration. Pavoni et al. (1975), in reviewing the work of Carpenter and Setter (1940), and Eliassen (1942) stated that the temperature of newly deposited waste increased quickly from 50 to 70 C. They remarked that this was the range of thermophile microorganisms. They also stated that the temperature ususally decreased slowly to 60 C and then fell rapidly to 29 C where it stabilized.

In an effort to stabilize the landfills, Pohland (1972, 1975) recirculated the leachate. For the experiment he used four test cells containing 1400 lbs of municipal waste packed at a density of 535 lbs/yd³. He added 250 gallons of tap water initially to start the production of leachate. Cell one was a control cell with no leachate recirculation, pH control, or sludge seeding added. Cell 2 recirculated the leachate only. Cell 3 recirculated the leachate with pH control (pH 6.8 to 7.4) only. Cell 4 had leachate recirculated, pH control, and raw sludge seed added. He found that the rapid stabilization was due to the increased anaerobic bacterial population growth. The pH control allowed more microbial stabilization than without it. The pH control also reduced the inorganic constituents.

Sonoma County Department of Public Works and Emcom Associates (1972 and 1973) constructed five municipal solid waste lysimeters at Sonoma County, California. This work was done for the purpose of determining the effect of liquids on waste stabilization. The waste was left intact and was not shredded. The type of lysimeters used and the results of the chemical determinations following two years of the study are presented below:

Cell A - A control cell to which no water was added. The leachate contained a low pH with a high volatile acid content indicating the pres-

ence of anaerobes. Methane was present in low instead of high concentrations indicating less than desirable methane-producing conditions. The metals analysis indicated that the levels were near the USPHS water standard: Zn $\leq$ 5 ppm; Pb $\leq$ 50 ppm; Hg $\leq$ 5 ppm.

Cell B - The Test Cell which was brought to field capacity immediately and to which no further additions were made. Anaerobic digestion produced high concentrations of volatile acids, low pH, and low CH₄ volumes. The metal content was similar to Cell A.

Cell C - Continuous flow of water through the cell. This produced vigorous anaerobic digestion of BOD, COD, and volatile acids. Methane was produced at an increasing rate in spite of the low pH. All metals were low but present in significantly high concentration except for cadmium, which was not present.

Cell D - Leachate recirculated. Active anaerobic activity occurred as indicated by the high volatile acid concentration and the low pH. The methane sharply increased in spite of the low pH (5.0).

Cell E - Cell brought immediately to field capacity with septic tank pumpings. No other liquid was added. The microbial seeding increased the anaerobic degradation. All leachate parameter concentrations increased with time, while the pH decreased and had an average of 5.0 during the second year. The heavy metal concentrations increased to levels equivalent to those of Cells A and B.

The results showed that Cell B had the highest fecal indicator levels. The fecal coliform levels dropped from 10^5 to 10^1 MPN/100 ml in six months and lower during the second year. The fecal streptococcal levels dropped from 10^7 initially, remained at 10^3 MPN/100 ml, and continued at the 10^2 level after another six months. Other high streptococcal levels came from cell C which dropped from 10^7 to 10^1 MPN/100 ml in nine months and those of cell D dropped to the same extent in five months. In cells other than B the fecal coliforms were present in insignificant levels while the fecal streptococci from all cells remained at greater than 10^1 MPN/100 ml for ten months.

4. Studies on Viral and Bacterial Survival in Landfills

The first study of significance on viruses in solid

waste was run by Peterson (1971). In an earlier section of this review she had reported finding poliovirus in baby's diapers in municipal waste. Peterson also placed poliovirus in a laboratory municipal waste lysimeter designed to determine viral survival and recovery rates. She found poliovirus in the leachate samples on the 10th and the 13th days following lysimeter construction. She typed these viruses for the poliovirus types and found that they both were poliovirus type 3, the vaccine strain type. However, when she added poliovirus to solid waste during the construction of the Boone County full scale landfill, she was not able to recover the virus from samples taken during a period of ten to 94 days. Thus, it would appear that viruses may possibly survive for only a limited time in landfills.

Following Peterson's work indicating that poliovirus could be found in municipal waste from landfills, and possibly in the leachate leaving the lysimeters and the landfills, several municipal waste lysimeter studies were initiated to determine the survival of viruses and fecal indicator bacteria in these waste facilities. The investigators included the work of Engelbrecht et al., (1974) from the University of Illinois, Cooper et al., (1974) from the University of California, Blannon and Peterson from the EPA in Cincinnati, Ohio, Glotzbecker (1974), and Novello (1974) from Scarpino's laboratory at the University of Cincinnati,

and Sobsey (1974, 1975, and 1978) from Baylor College of Medicine and from the University of North Carolina.

Further studies on the survival of viruses and fecal indicators were made by Engelbrecht et al. (1974). He used a 10 x 5 ft steel lysimeter to simulate a sanitary landfill. In this lysimeter they used 3400 lbs. of Cincinnati, Ohio solid waste containing 11% food wastes, 8% garden wastes, and 3% baby's diapers. The waste was shredded and stored. During the storage but previous to its placement in lysimeters, aerobic decomposition began and the temperature rose to 149 F. At this time the waste was placed in cold storage. Following this it was shipped to Illinois where they packed it in the experimental lysimeter. At each six inch level poliovirus I LSc was added. Deionized water was added until the field capacity was reached (36 days after construction) and leachate was produced. The temperature inside the lysimeter was 104 F for 4 to 20 days and averaged greater than 80 F up to 60 days. The pH varied from 5.25 to 5.63. Both the conductivity and the COD increased all during the study. The conductivity varied from 13,700 to 20,000 and the COD varied from 49,300 to 69,000 mg/L. In the 120th day of the experiment, the total coliforms dropped to 6 CFU/100 ml and the fecal coliforms dropped to 2 CFU/100 ml and the fecal streptococcus dropped to 1×10^1 CFU/100 ml of leachate. Then the coliform count dropped below the detec-

tion limits while the streptococci remained steady up to the 140th day, the final day of the experiment. Of the 10^{10} poliovirus added to the lysimeter during the construction, no viruses were isolated from the leachate samples. The authors speculated that the high temperature (149 F) attained initially and prior to being placed in the lysimeter, was a contributing factor to the low fecal indicator levels. Finally, Engelbrecht et al., showed that leachate contained an inactivation factor. By adding fecal coliforms, fecal streptococci, and Salmonella to a 277 day old leachate sample they determined that at 22 C, and at pH 5.3 the Salmonella was the most stable, while at pH 7.0 the fecal streptococci was the most stable. The die-away for all three groups was more rapid at pH 5.3. Finally they found that these groups survived to a greater extent in buffer than in leachate. They concluded that there was an inactivation property for viruses, fecal coliforms, fecal streptococci and for Salmonella, the latter demonstrating less sensitivity than the other groups.

Also using lysimeters to study microbial survival, Cooper et al., (1974) was concerned with the large amounts of untreated human and animal wastes placed in landfills with municipal waste containing high levels of enteric bacteria and viruses which may leave the landfills or open dumps and transfer to ground or surface waters. In his

study, he compared the impact of added disposable diapers on sanitary landfills and open dumps. For this purpose they used open (no soil on top of the waste) and closed (with 4 inches of soil on top of the waste) lysimeters. Sixteen circular lysimeters were used of which 14 measured 5 ft X 3 ft and two larger ones measured 10 ft X 3.5 ft. These lysimeters were filled with municipal solid waste, soiled diapers and poliovirus type 1 (LSc). Their experiments ran for 20 weeks. The results during the first 20 weeks indicated a difference between open and closed lysimeters. The closed lysimeter had decreased fecal indicator levels in the leachate ranging from 10^5 to 10^1 most probable number (MPN)/100 ml for both total and fecal coliforms and levels of 10^7 to 10^5 MPN/100 ml for fecal streptococci. For the open lysimeters both total coliform and fecal coliform levels decreased from 10^6 to 10^3 MPN/100 ml and fecal streptococci decreased from 10^9 to 10^6 MPN/100 ml. A comparison of the chemical characteristics indicated that the total solids, COD, volatile acids, and total nitrogen levels in the leachate were lower in the open lysimeter than in the closed lysimeter, indicating a slower organic decomposition process in the former unit. They recovered viruses only from the closed lysimeter during the second and third week. In summary, the significance of the work was that the viruses and the coliform levels declined rapidly in the simulated

sanitary landfill. Also, these results suggested that the presence of oxygen in the open lysimeter simulating open dumps had an effect upon slowing the waste decomposition and upon the increased survival of the microorganisms.

In a change from lysimeters to field sites, Blannon and Peterson (1974) examined the leachate from a full-scale landfill (Boone County experimental landfill). The landfill was constructed in June 1971 and the tests began when the leachate appeared. The fecal coliform counts varied from 10^6 for September to 10^3 for December and to $10^1/100$ ml for June of the following year. Similarly the fecal streptococci varied from 10^7 in September, to 10^5 for December to 10^4 for June of the following year, i.e. MPN/100ml of leachate. Blannon and Peterson calculated the fecal coliform/fecal streptococci ratio using these MPN results. This ratio was originally described by Geldreich and Kenner (1969) who found that a number of greater than 4.0 indicated that the pollution was caused by human fecal material, while a number of less than 0.7 indicated that the pollution was caused by livestock or poultry. Blannon and Peterson demonstrated a 6.2 ratio in September, two months following the landfill construction, indicating that human waste was present in the municipal solid waste placed in the landfill. Blannon and Peterson also characterized the streptococci present. They examined 60 strains, 35 of which (58.3%) were

Streptococcus faecalis var. liquefaciens, and 5 (8.33%) were atypical streptococci (found on vegetables). Thus, 66.7% of the streptococci had no sanitary significance. The rest of the strains, 33.3% included S. faecalis biotype II and III (12 strains), S. durans (3 strains), and S. equinus (5 strains) were from warm blooded animals. Blannon and Peterson concluded that leachate contamination may pollute the soil, recreational waters, and ground water, and "may pose hazards to the public health".

Field studies were also made by Glotzbecker (1974) and Novello (1974). Glotzbecker examined two landfills. First he examined the leachate from an active commercial landfill using the Standard Methods (1974) most-probable-number (MLD/100 ml) test. He found that the total coliform, fecal coliform and the fecal streptococci levels ranged from 10^2 to 10^3 ; 10^1 to 10^2 ; and 10^1 to 10^3 MPN/100 ml, respectively. Second, he examined the three year old Boone County landfill in 1974 and found that the fecal indicator levels were varied from <3 to 10^2 for the total coliforms, <3 for the fecal coliforms, and varied from <3 to 10^2 MPN/100 ml for the fecal streptococci in leachate from the upper pipe (at the bottom of the landfill). In the lower pipe (in the clay liner beneath the landfill) the fecal indicator levels were <3 to 10^4 for the total coliforms, <3 to 10^2 for the fecal coliforms, and 3 to 10^2 MLD/100 ml for the fecal

streptococci. Also at the Boone County landfill were three municipal waste lysimeters (12 X 6 ft) and a small landfill (28 X 28 X 8 ft). The total coliform, fecal coliform and fecal streptococcal levels in leachate after two years were < 3 , < 3 , and 10^2 to 10^3 MPN/100 ml, respectively. In addition, he did survival studies using a commercial landfill. He added cultures of Escherichia coli and Streptococcus faecalis to the leachate and incubated the mixtures of both at 10 C. After 35 days there was a 10% survival of the E. coli and 30% of the streptococci. He concluded that the fecal indicator bacterial survival in different landfill leachate was variable. Novello (1974), (Glotzbecker and Novello, 1975) studied viral survival in landfill leachate. He used the poliovirus type 1 Mahoney (LP) RKP. He reported that it was necessary to use EDTA in the assay procedure for viral recovery following the incubation of viruses and leachates at 10 C. Also, the viral recovery was inversely related to the incubating temperature. In addition, viral survival was greater in leachate from the Boone County full-scale landfill than from a large commercial landfill when the leachate and virus were incubated at temperatures of 10 C and 20 C. This is shown by the following data. After storing the leachates and viruses at 10 C for 90 days, the percentage survival of virus was 36 for the leachate from Boone County and 8.9 for the leachate from the commercial landfill.

Sobsey et al., (1975) added poliovirus 1 and echovirus type 7 to a laboratory scale municipal solid waste lysimeter. No viruses were detected in the leachate over a four month period. The lack of viruses appearing in the leachate were due, they concluded, to two actions: one, to viral adsorption by the municipal waste, and, second, to viral inactivation.

Riley et al., (1977) studied leachate from a municipal landfill in Washington state. They indicated 38 isolates from leachate which had seeped from the top soil of the landfill: Pseudomonas, Flavobacterium, Bacillus, Alcaligenes, Sarcina, Xanthomonas, Streptomyces and Penicillium species. The most probable number (MPN) presumptive coliform test was $10^2/100$ ml.

Cammeron and McDonald (1977) added leachate and septic tank pumpings together in various ratios: 50:50; 10:90; and 0:100. At the end of one day the 50% leachate and 50% septic tank pumpings (raw sewage) had no detectable coliform levels, in the 10:90 ratio the fecal coliform levels (most-probable-number/100 ml) decreased to less than 10^2 MPN/100/ml in 22 days, while the raw sewage had a total coliform level of 10^7 and fecal coliform level of 10^4 MPN/100 ml at the end of 22 days. These results indicated to the authors that the leachate was toxic to the total coliforms and the fecal coliforms. Yet, they noticed that there

was abundant gas production demonstrating that some microorganisms were thriving. They concluded that it was possible that there could be pathogenic microorganisms present, and that the fecal indicator bacteria were killed and could not, therefore, indicate pathogen presence. These results indicated a need for a more suitable indicator system for landfill leachates. In addition they found evidence that leachate contained a bacterial inhibitory substance. They placed ether concentrated leachate on a paper disc, 12.5 mm in diameter, and placed this on a natural agar plate streaked with Escherichia coli. Following the incubation there was an inhibitory zone around the disc (no growth) which was 33 mm in diameter. Paper chromatography indicated that the concentrate contained propionic, n-butyric, n-valeric, n-caproic, isovaleric, and acetic acid. The stripped leachate no longer inhibited coliforms.

In more recent investigations Sobsey (1978) examined the leachate from 21 active and inactive landfills. These landfills varied in age from 1 to 15 years. He was able to find viruses from only one landfill leachate sample. He concluded that the enteric viral concentrations landfills were so low in leachate seeping from landfills that they would be diluted by the ground and surface waters. Thus, the viruses could not constitute an environmental or a public health hazard. On the other hand his fecal indicator

assays showed at times high levels in the leachate. Overall, these results indicate little if any distinction between the active and inactive landfills. However, one landfill, inactive for fifteen years, containing total coliform and fecal coliform levels of 1.1×10^3 MPN/100 ml of leachate is unusual. It was remarkable that these microorganisms survived with high levels for fifteen years indicating, of course, the continued survival of human pathogens.

D. MICROBIAL STRESS

1. Background

The results of the microbial enumeration of leachate depended upon the methods used by the investigators. Included in these methods would be a description of the type of media used, and incubation temperatures. These methods, taken from other disciplines such as water, food, or clinical microbiology, were probably adapted in some way when they were used to measure the presence of microorganisms in the soil, sewage, and leachate. It is these adaptations that are required to enhance the growth of stressed bacteria and this section of the review will include the description of the conditions used to overcome that stress.

2. Environmental Conditions Which Stress Bacteria

When toxic and stressful conditions may be indicated, less than expected concentrations of microorganisms are

present in an environmental sample. Low bacterial numbers have been found in leachate studies, particularly those from lysimeters (for example, Engelbrecht et al., 1974). Postgate (1967) stated that "bacteria subject to stress become hypersensitive to secondary stresses...". Bacteria may become stressed, i.e. the leachate causing the primary stress, and the selective media the secondary stress. When the growth, enumeration, and identification of microorganisms from leachate are hampered by stressful conditions, then there will be fewer numbers of bacteria and fewer species will be isolated.

Other environmental sites where primary stresses may be found include sewage sludge, river water, and soil. Wang, et al. (1956) found that Shigella flexneri was adversely affected by sterile sewage. They showed a decline in microbial numbers recovered on SS agar as compared to an agar containing no inhibitory constituents. Furthermore, this decline was reversed by extended incubation, and by adjusting the pH to 7.5 using phosphate buffers, followed by the addition of NaCl or KCl. Rose and Litskey (1965), in their examination of river water, were able to double the membrane filter count of streptococci by including a pre-incubation period (3 to 18 hrs.) at 35 C and by using an enrichment broth prior to further incubation on m-entero-

coccus agar. The enrichment broth contained peptone, yeast extract, and casitone. Bissonnette et al., (1977) placed their membrane filter chamber containing Escherichia coli in river water for three days to determine the extent of the environmental stress on the microbial cells. They used the membrane filter to determine survival. The microorganisms were stressed causing the counts to decrease on selective media, m-FC agar, as compared to the counts on enriched agar. The inhibition was reversed two-fold by a preincubation of the membrane filter for 2 hours at 35 C on trypticase soy agar supplemented with yeast extract and glucose prior to placing the filter on m-FC agar at 44.5 C for 24 hours. Rose et al., (1975), using the two layer agar procedure (lactose agar poured over m-FC agar in a petri dish) for incubation of membrane filter bacteria, were able to increase the recovery of the stressed fecal coliforms from river water by 93%. In an analysis of dry and moist soil, and rabbit droppings from the top of Stone Mountain, Georgia, Thomason et al., (1977) were able to increase the Salmonella isolations by 25%. Their procedure included the addition of an enrichment broth, i.e. inoculation in buffered peptone water for 24 hours prior to inoculation into tetrathionate broth.

The above experimental work demonstrated that the microorganisms in the environment become stressed. The

stressed condition means that microorganisms frequently do not grow on selective or inhibitory media unless this injury is repaired. In order to bring about this repair, growth conditions must be improved.

Among the many reasons for the lack of pathogen or pathogen-indicator growth on selective or inhibitory media is that the microorganisms are dead or not present in a particular sample (Hendricks, 1971). Others include the lack of certain ions (Geldreich, 1976) and metabolites from the media (Iandolo et al., 1965) bringing about a starvation effect (Wu and Klein, 1975). The microorganisms may have been subjected to inhibitory or toxic agents (Wang et al., 1956; Molina et al., 1972) or to other microbial interferences which could change their metabolic patterns (Allen et al., 1976). They may have survived ultraviolet chlorine disinfection (Heinmets et al., 1954b; and Braswell and Boardley, 1974) and other disinfectant action (Scheusner et al., 1971). Lastly, the microorganisms may have been subjected to physical stress such as heat, freezing, drying and radiation (Sinskey & Silverman, 1970; Ray et al., 1971; Ray & Speck, 1972a, 1972b, and 1973; Adams, 1974; Beuchat and Lechowich, 1968; Brewer et al., 1977; Duncan et al., 1972; Smolka et al., 1974; and Klein & Wu, 1973).

Both starvation and metabolite removal or addition may decrease microbial survival. For example, Wu and Klein

(1975) studied the effect of the addition of glucose and a subsequent warming stress on starved Escherichia coli and Aeromonas sp. The latter microbe was taken from the environment. The authors starved the Escherichia coli for three weeks, added glucose for 1 hr. and then stressed the bacteria in a waterbath at 49.5 C for 2 minutes. The E. coli decreased in numbers over those not so stressed with the glucose addition. On the other hand, the Aeromonas sp. similarly treated was not adversely affected by the addition of glucose. Wu and Klein concluded that the starvation was the primary stress and that the addition of glucose increased the effect of the warming stress. They concluded that the glucose was the secondary stress, thereby decreasing the microbial levels. They suggested that this could be the reason why Aeromonas may be found in the environment and why E. coli was excluded from aquatic environments. Furthermore, Iandolo et al., (1965) found that Staphylococcus aureus growth was depressed in the presence of Streptococcus diacetelactic. When nicotinamide was added to the culture, growth resumed. Also, Molina et al., (1972) found digested sludge to be toxic to E. coli. Tryptone added to sludge, reversed this toxicity. Similar results were reported by Geldreich (1976). In an experiment simulating storm runoff, 0.1% magnesium ions gave Streptococcus faecalis a longer survival time than did sodium and calcium ions.

Related to this is the work of Allen et al., (1976). They analyzed water in water mains at extended points from the treatment plant and found low total coliform MPN counts with high plate counts. By inoculating all lauryl sulfate broth tubes, regardless of whether or not gas was produced into brilliant green bile tubes, it was found that gas was produced in more brilliant green bile tubes than there were originally positive lauryl sulfate tubes. This indicated to them that the high count interfered with usual metabolic activity, preventing gas production in lauryl sulfate broth. Blind transfers from lauryl sulfate into EC broth also produced higher fecal coliform counts.

The disinfection activity of chlorine, or quarterary ammonium compounds in the laboratory or in environmental waters injure but do not kill all exposed E. coli and other test microorganisms as determined by the addition of certain nutrients which aid in revitalizing these stressed bacteria. Namely, Heinmets et al., (1954a) exposed E. coli to 1 ppm chlorine. He was able to revitalize "sterilized" cultures by the addition of sodium citrate, oxalacetic, lactic, and malic acids. Braswell & Hoadley (1974) subjected Escherichia coli in sterile secondary effluent to chlorine as NaOCl (2 mg/l) for 20 minutes at 21 C. The membrane filtration procedure was used to count these bacteria. Membrane filters placed on m-FC medium showed no viable counts while those

placed on trypticase soy plates varied from 10^1 to 10^3 ml. The MPN titer using lactose broth was $< 10^1$ /ml.

A great deal of work has been done on the stress effect of freeze-drying of microorganisms (Ray et al., 1971; Ray & Speck 1972a, 1972b, 1973). They examined thawed freeze-dried Salmonella anatum. Seventy to 90% of the survived cells in nonfat dried milk were injured. The injured survivors failed to grow on selective (desoxycholate) agar but did form colonies on nonselective media. They found that death and injury of the cells were accompanied by the loss of intracellular materials. They were able to bring about the growth of these injured cells after a one hour repair period at 25 C. The cells still showed some signs of injury as they had an increase in the lag period and the cells grew at a slower rate. Also from other studies on frozen food (whole eggs) with injured cells, Escherichia coli demonstrated increased bacterial counts following a one hour repair period at 25 C in trypticase soy agar containing yeast extract as compared to the growth on selective agar (violet red bile).

Injury to microbial cells is also produced by low levels of heat and by drying. The heat stress of Streptococcus faecalis was studied by Beuchat & Lechowich (1968). Heat injury at 60 C was demonstrated by the lack of growth in high salt concentrations compared to growth in media

without salt. The degree of injury varied directly with the length of time the cells were heated. Brewer et al., (1977) described a similar heat effect on cells of Staphylococcus aureus. Mossel & Ratto (1970) detected sublethally impaired Enterobacteriaceae in dried foods, feeds, and drugs. Initially, they found that buffered glucose brilliant green bile broth as the enrichment medium and violet red bile glucose agar as the identifying medium gave low microbial counts, however, by use of a pretreatment, additional samples became positive. The best results were obtained when food samples inoculated into buffered tryptone soya peptone broth was incubated at 19 to 25 C for 1 to 6 hours, followed by inoculations of the culture on the selective media listed above. Without the use of this technique slightly under 40% of the positive samples would have been missed.

The stressful conditions listed above may kill or injure microorganisms. Some of the characteristics of these stressed or injured bacteria include an extended lag time (Sinskey and Silverman, 1970), the leakage of cellular material with the release of peptides or RNA (Sinskey & Silverman, 1970; Ray & Speck, 1972a; and Beuchat & Lechowich, 1968), increased nutritional requirements (Brewer et al., 1977; Geldreich, 1976; Thomason et al., 1977; Rose et al., 1975; and Bissonnette et al., 1977), greater susceptibility to antibiotics (Sinskey & Silverman, 1970; and Barach

et al., 1975), extended incubation periods or slow growth (Goff et al., 1972), growth inhibition in selective media (Bissonnette et al. 1975, 1977; Rose & Litskey, 1965; Scheusner, et al., 1971; and Ray & Speck, 1973a), growth inhibition by bile salts or sodium chloride (Bauchat & Lechowich, 1968; Goff, 1972; Smolka et al., 1974; Hartman et al., 1975; and Brewer et al., 1977), and an increased susceptibility to temperature at 50 C during the pour plate process (Speck et al., 1975; and Klein & Wu, 1973).

3 Laboratory Cultivation Methods Designed to Overcome Stress

Because the membrane filter (MF) procedure has many advantages over the most probably number (MPN) procedure for enumerating coliforms and streptococci, it has been used extensively; however, it does give lower results on occasion when compared to the pour plates, and the MPN procedure for raw sewage and stream environments (Rose et al., 1975; Geldreich, 1976; Braswell and Hoadley, 1974; Bissonnette et al., 1977). Also, Glotzbecker (1974) compared the MF and the MPN procedure in the analysis of total coliforms, and fecal streptococci from landfill leachates samples. The MPN consistently gave the higher results. Both Braswell and Hoadley (1974) and Geldreich (1976) stated that the MPN test was preferred over the MF for fecal coliform determination of chlorinated effluents. Nevertheless, Rose et al. (1965)

increased two-fold the recovery numbers of fecal streptococci on membrane filters by the addition of peptone-yeast extract-casitone enrichment broth to the filters for an incubation period lasting from 3 to 18 hours followed by the transfer of the filter to m-enterococcus agar (over that of the m-enterococcus agar alone) for the remainder of the 48 hour incubation period at 35 C.

Later, Rose et al., (1975) used the two agar layer to improve MF counts. They poured the selective m-FC agar into the bottom of the petri dish and added a lactose agar layer on top of the first layer. The filter was then placed upon the top layer. The plate was incubated at 37 C for 2 hours and then continued at 44.5 C for 22 hours. The growth of fecal coliforms increased 93%. Thus, stressed bacteria were able to initiate growth on the nonselective top agar layer at a lower temperature (35 C as opposed to 44.5 C) before the growth-limiting m-FC components diffused into the lactose agar layer and inhibited bacterial growth. Similarly, Bissonnette et al., (1977) were able to improve the numbers of stressed E. coli after a three day exposure to river water by adding a preincubation step. They placed the MF filter upon non-selective media for 2 hours at 37 C and then on selective media, for 24 hours. The microbial count was increased by two-fold.

Apparently, many agents, both simple and complex, and various conditions are able to aid in restoring the growth of viable stressed microorganisms. Among the simple agents used have been the Krebs cycle compounds. The work of Heinmets (Heinmets, 1953; Heinmets et al., 1954a, 1954b) demonstrated that stressed or "killed" bacteria could be revitalized by carbohydrate decomposition products. In 1953 Heinmets used ultraviolet irradiated E. coli B/r cells. Noting that some cells after a photo-chemical reactions were capable of self-recovery, Heinmets set about to see what nutrients, if any, could reverse the ultraviolet damage to bacteria. He used the products of glucose metabolism, especially that of the Krebs cycle. These were added to a nonselective medium. Pyruvate was by far the better agent to reverse the E. coli stress. He continued the work to include microbial stress by disinfection agents such as H_2O_2 , chlorine, zephiran, and ethanol, and by heat. His greatest recovery of stressed microorganisms came with the addition of several Krebs cycle components to the growth medium. Closely related to this work is the work of Ray and Speck (1972a, 1972b, 1973). They studied E. coli stressed by the freeze-dried process. The enumeration of cells on trypticase soy-yeast extract-deoxycholate agar resulted in the loss of 90% of the cells as compared to 50% on agar without the inhibiting agent. Repair of the stressed cells

occurred by subjecting them to a repair medium consisting of trypticase soy-yeast extract broth and with an incubation period at 20 C for 2 hours prior to the enumeration upon the complete agar medium. The addition of Mg^{++} and pyruvate or KH_2PO_4 for 2 hours at 20 C facilitated the repair. The repaired cells were then able to grow on selective media (Ray et al., 1972a). They also demonstrated that ATP added in the same manner was able to bring about repair (Ray and Speck, 1972b). On the other hand, Sinskey and Silverman (1970) found that trypticase, casamino acids, and peptone were superior to glycerol in initiating cell growth of freeze dried E. coli cells.

Kenner and Clark's (1974) work supports the view that digested metabolites will aid microbial growth. They used heat-killed Salmonella paratyphi A in broth culture to aid the initiation of Salmonella growth from the environmental sources, such as storm water runoff and municipal wastewater. Their enumeration procedure, most-probable-number technique, included incubating the sample in dulcitol-selenite broth followed by streaking the cultures on xylose lysine deoxycholate agar for the isolation and identification of Salmonella strains. They found that the addition of the heat-killed culture to dulcitol selenite broth increased Salmonella isolations from wastewater effluent from 57% to 74%. The digested metabolites probably contained some of

the same low carbon compounds as were used by Heinmets et al., (1954a). Larger compounds, also, aided in the repair; lactose and sucrose were used by Ray et al., (1971) and lactose with buffered peptone water were used by Thomason et al., (1977). Nonselective media were used to repair stressed cultures (Mossel & Ratto, 1970; Hartman et al., 1975; Rose et al., 1975). A repair period, using these reagents in enriched media or with an addition to selective media, have increased the growth of stressed microorganisms. Various repair times have been used; Ray et al., (1971) used 1 hour; Rose et al., (1965) used 3 hours; Mossel and Ratto (1970); found that 1 to 2 was best and Thomason et al., (1977) used 24 hours. Apparently, a variety of repair agents and repair periods are effective to decrease the effect of stress on environmental bacteria.

The study of stress and growth enhancement of Gram-positive cocci has supported this work on Gram-negative rods. Rose and Litskey (1965) placed the membrane filter used for river water sampling upon peptone - yeast extract-casitone enrichment medium for 3-18 hours at 35 C prior to its being placed upon m-Enterococcus agar. The yield of the streptococci colonies doubled over those on m-Enterococcus agar without the intermediate step using an enrichment medium. In addition, magnesium ions must be of use to microorganisms as Postgate (1967) found that magnesium ions

were important to the survival of stressed microorganisms. Also, Geldreich (1976) had shown that magnesium ions (0.1%) in storm runoff had a greater effect on the survival of Streptococcus faecalis than had calcium or sodium ions. On the other hand, Beuchat and Lechowich (1978) found that stressed streptococcal growth decreased after heat at 60 C by the addition of 0.1 to 0.4 moles of magnesium ions to all-purpose tween broth incubated 30 C for 24 hours. Finally, Brewer et al., (1977) were able to increase the growth of heat stressed Staphylococcus aureus in trypticase soy broth containing 10% NaCl by the addition of pyruvate and catalase in the most-probable-number procedure.

Microorganisms from the aquatic environment are susceptible to mild transient heat contributed by the agar during the pour plate procedure (Klein and Wu, 1973). The colony counts were lower compared to the spread plate technique. Consequently, when enumerating microorganisms from the environment the standard plate count should never be used as presently described. Also important is that microorganisms from the environment are usually starved as nutrients are not always available. This starvation represents the primary stress. Agar heated to 50 C, for use in pour plates, represents the secondary stress (Postgate, 1967). Consequently, the injured microorganisms are stressed twice and as a result produce fewer colonies than expected.

Speck et al., (1975) reported on a procedure to repair coliforms injured by freezing. This involved the inoculation of injured coliforms by surface plating on trypticase soy agar (repair medium); an incubation period at 25 C for 2 hours followed by the addition of a violet red bile agar overlay (inhibitory medium), and the continued incubation at 37 C for 24 hours.

In conclusion, it appears that most bacteria of human origin, isolated from the environment, have become stressed or injured in some manner. This is of prime importance when the microorganisms so stressed are pathogenic and do not grow out on differential media to indicate their presence.

The repair of these pathogenic bacteria from environmental sources and their measurement on differential media will give a better indication of bacteria concentrations than is now the case.

E. EVIDENCE FOR GROUND WATER CONTAMINATION BY
LEACHATE MICROORGANISMS

The study of municipal landfills and lysimeters in and of themselves is not totally valid unless one considers their environmental impact. They may produce large quantities of leachate containing various chemicals and microorganisms. One important result of this pollution is the potential for extended ground water contamination by landfill leachate. Furthermore, this pollution, already established for chemicals in the environment, has not been established for microorganisms or their products. Therefore, it is the purpose of this portion of the review to review the evidence for such a possibility.

The evidence for ground water contamination by leachate microorganisms is presented here in five steps.

1. Fecal indicator microorganisms have been found to survive up to fifteen years in landfills.
2. Bacteria have been found to survive for long periods of time in the soil.
3. Some geological formations weather quickly or are dissolved by the acid from the leachate and leave holes in the soil and rocks through which leachate may find easy access to the ground water.

4. The leachate, with decomposed waste components, have been found in ground water at distances greater than one mile from the landfills.
5. Viruses and bacteria can filter down through the soil to the ground water, and these microorganisms having reached the ground water can travel for various distances in the direction of the ground water flow. An elaboration of these points of evidence is presented below.

First, leachate from landfills (and lysimeters) carrying fecal indicator bacteria have been found to be present consistently in less than one year old landfills and sporatically in greater than one year old landfills. As these results were just described in detail in a previous section, only representative data will be presented here. From the less than one year old landfills (lysimeters), specifically those that were three months old, the leachate at concentrations of MPN (or CFU)/100 ml of leachate, contained fecal coliform levels varying from 10^1 to 10^3 MPN/100 ml, while the fecal streptococci concentrations varied from 10^1 to 10^7 MPN/100 ml (Engelbrecht et al., 1974; Cooper et al., 1974; and Blannon and Peterson, 1974). In landfills over a one year period Blannon and Peterson (1974) while examining the Boone County landfill, found that the fecal indicator levels diminished only slowly such that the fecal

coliforms (MPN/100 ml of leachate) originally present at 10^6 decreased to 10^1 , while the fecal streptococci levels (also at MPN/100 ml) dropped from 10^7 and remained at a steady 10^4 . Also, Carpenter and Setter (1940) reported that the Escherichia coli levels in leachate from a less than two year old landfill averaged 10^5 most probable number per 100 ml. In addition, the older inactive landfills continued to have significant fecal coliform levels. Sobsey (1978) analyzed eight inactive landfills for coliform levels (MPN/100 ml leachate) and found that four of the landfills between two and eight years old had levels of < 3 MPN/100 ml, four landfills between two to ten years had levels of 11 to 2.4×10^2 MPN/100 ml and the landfill fifteen years old had a level of 1.1×10^3 MPN/100 ml. It would appear, therefore, that the potential for microbial pollution begins immediately upon the deposition of the waste and continues for extended periods of time up to 15 years or more.

Second, microorganisms have been found to survive for long periods of time in the soil. Accordingly, Rudolf et al., (1950, 1951) in a review of fecal pathogen survival in soil, water and sludge concluded that while Eberthella typhosa, (Salmonella typhi) generally, lived less than 100 days in soil, they have survived longer than 2 years in freezing moist soil. The conditions reported as most favorable to this bacterium was a cold temperature, moist soil,

and a slightly alkaline pH. Further, Escherichia coli appear to survive longer in soil than E. typhosa. Vibrio cholera survived greater than 11 days in sea water. The authors concluded the review by saying that of intestinal microbes, pathogenic and non-pathogenic, E. typhosa, E. coli, and Mycobacterium tuberculosis appear to be the most resistant to natural conditions (vegetables, soil, etc.) outside the human body. In addition, Geldreich (1972) in a review of water-borne pathogens pointed to the extended Shigella persistence at -45 C in feces and soil for 145 and 135 days, respectively, in Siberia. Also, cultures taken from the arctic environment and tested showed that virulence was not lost, but biochemical characteristics had changed due to the low temperature. Hutchenson (1974) listed the conditions which affected pathogen survival in the environment as: 1), the nature of the suspending medium, such as feces, sewage, soil, and water, 2), the physical environment such as the temperature, or the light intensity; 3), the presence of other biological agents such as bacteria, phages, protozoa, and other predatory pollutants; and 4), the presence of biocidal agents such as natural antagonists from biological forms or from chemical pollutants. Finally, he gave the results of microbial survival: Salmonella, four months in cold weather from feces, cesspools and subsoil drains and 443 days in distilled water; Shigella for 135

days in soil at -45 C; coxsackie for 272 days in distilled water; and poliovirus for 150 to 170 days in moist soil at pH 7.5. Further examples of microbial survival in the environment are given in Table 5.

Third, acid leachate may enlarge porous rock formations such as fissures, faults, and joints, and allow unrestricted leachate contamination of the ground water. We may conclude that arid parts of the United States produce small amounts of landfill leachate but in the temperate zones, especially in the eastern United States the rain is 35 inches or more and quantities of leachate are produced (Mann, 1976; and Brunner, 1977, personal communication). As stated earlier, municipal solid waste contains organic material, including fecal wastes. This fecal material included the fermentative microorganisms which degraded the organic waste with the production of acids and carbon dioxide. During the early stages of the landfill, the carbon dioxide was produced in large amounts until the oxygen is utilized, and then it decreased to some degree. This carbon dioxide dissolved in the leachate and affected the pH (Coe, 1970). The pH eventually lowered to an average of 5.6 to 4.8 within the first four years (Walsh et al., 1979). The pH probably had two important effects on microbial survival. The low pH, and the increased presence of metal ions could decrease microbial levels (Streng, 1976). These metal ions

TABLE 5. The Survival of Microorganisms in the Environment

Microorganisms	Contam- ination Source	Site	Survival Time	Reference
<u>Salmonella</u>	Sludge	Grass	40-58 wks.	Love, (1975)
	Manure slurry	Soil	12 weeks	Elliott & Ellis, (1977)
	Culture + feces	Soil	8 weeks	Temple, et al., (1980)
	Culture + feces	Soil	42 days	Zibilisks & Weaver, (1978)
	Feces	Soil	Several Months	Hoadley and Goyal, (1976)
Fecal Coliforms	Raw Waste	Soil	10 months	Johnson & Urie, (1976)
<u>Escherichia Coli</u>	Culture + Feces	Soil	8 weeks	Temple et al., (1980)
Fecal <u>Strepto- coccus</u>	Raw Waste	Soil	1 month	Johnson & Urie (1976)
<u>Mycobacterium bovis</u>	Culture + Sludge	Vegetables	35 days	VanDonsel & Larkin (1971)
<u>M. tuberculosis</u>	Sludge	Soil	15 months	Jensen, (1954)
Coliphage	Digested Sludge (Anaerobic)	Land Application	11 days	Sagik et al., (1980)
Enterovirus	ND ^a	Soil	8 days	Burge and Marsh, (1978)
Poliovirus	ND ^a	Polluted Water	20 days	
Poliovirus 1	Culture + sewage sludge	Soil	96 days	Tierney et al., (1977)
Poliovirus	Culture + septic tank liquor	Soil	180 days	Yeager & O'Brien, (1979)
Poliovirus Coxsackievirus	Secondary effluent	Cypress Dome	28 days	Wellings et al., (1975)

a = Not Determined

were produced during the anaerobic phase of waste digestion, but their distance of travel in the leachate from the landfill was not determined (Rule, 1979). When this leachate moves from the landfill to the substrate below the surface of the earth, it may move downward in the zone of aeration, through the zone of saturation and on to the aquifers. The distance the leachate travels is dependent upon the soil, and bedrock. Soils that retain the leachate contain clay and loams. For example, nitrate pollution from a barnlot source moved through 200 feet of silty clay in 20 years (Walker, 1973a, 1973b). On the other hand shale, limestone, and dolomite may contain fissures, fractures and joints which absorbed the leachate and was dissolved by the acids to produce small channels. These small channels may increase in size with use and allow increased leachate flow (Deutsch, 1965; Salvato et al., 1971; Brown, 1964; and Faulkner and Pascale, 1975). This corrosion is due to the increased acids caused by the presence of excessive free carbon dioxide, which extracts the metal ions from metal objects. He states that free carbon dioxide in excess of 20 ppm will attack iron, steel, and concrete to the degree that protective measures must be added. This acidity also acts to release the CaCO_3 from concrete, and limestone. This increase in the carbonates, bicarbonates, and hydroxides increases the alkalinity which makes drinking water unpalatable to consumers (Coe, 1970).

The escape of leachate from landfills may bring about chemical changes in the environment. Weaver (1964) stated that carbon dioxide and ammonia produced from the decomposition of the organic matter were the most significant products produced. The CO_2 was found to increase the hardness of the leachate and the ammonium ions which on oxidation were found to increase its nitrate content. This caused the minerals to increase 20 times higher than the ground water concentrations, and the ammonium-nitrate levels to increase 10,000 times. Finally for each acre-foot sanitary landfills leached approximately 3.9 tons of bicarbonate per year. Furthermore, Walker (1973) related that both chemicals and microorganisms from garbage dumps will move through sand and gravel aquifers, shallow limestone or dolomite aquifers into the connected cracks, joints, and fissures seen in these bedrock formation. He also said that microorganisms will be removed from the polluted leachate flow by fine-grained soil, but not the chemical waste. These chemicals will continue on to the superficial ground water reservoirs. This was supported by the work of Deutsch (1965). On the other hand, Zanoni (1972) in his review stated that the landfill pollution to ground water was localized, and that the leachate was diluted as it passed through the ground water. The leachate as it passed through the soil was attenuated by dilution, adsorption and micro-

bial degradation. He also stated that the drinking wells should be at least 1000 feet from landfills.

Leachate may travel long distances in sandy soil. Kimmel and Braid (1974) described the contamination of Long Island ground water by two landfills 27 and 41 years old which have contaminated ground water plumes extending 10,600 and 5,000 feet, respectively, from the site of departure. In addition, the plumes sank in to the bottom of the aquifer to depths of 70 to 170 feet, respectively. In another study, concerning a sandy soil site, Berk and Yare (1977) were searching for possible contamination in water supply wells, derived from an industrial process water containing sodium chromate and sodium hydroxide which had already caused ground water pollution along the New Jersey coastal plain. They made electrical resistivity surveys using 100 ft probes to measure the variation in ohms and thus measure the vertical and lateral distribution of the contamination. Their results demonstrated that the fresh water/contaminated water interface had traveled greater than one-half mile (3,000 ft) down gradient. This work was supported by water assays made from newly dug test wells.

Fourth, the leachate from sanitary landfills with decomposed waste components have been found in ground water at distances greater than one mile from the landfill. Coe (1970) used sulfate and other parameters to measure the

landfill leachate pollution from monitoring wells at selected positions. He found the highest pollution at 4,000 feet well down gradient from the pollution point, and at a further well one mile away from the landfill he found a 31% increase in pollution. Coe warned that leachate polluted ground water may cause corrosion. He stated that the carbon dioxide produced by the decomposition of the landfill waste increased the hardness and the bicarbonate. He stated that the water may become corrosive. This change was dependent upon the pH. Moreover, the addition of leachate to the ground water did increase the organic matter and the mineral constituents due to the extracted materials from the refuse. Finally, he stated that the landfills may exist for years before the effects on the ground water are detected.

Reports vary in the lateral distances that bacteria and viruses may travel. Shaub and Sorber (1977) isolated enteroviruses and F_2 bacteriophages from a well 60 ft (18.3 m) deep and a horizontal distance of 600 ft (183 m) from the application zone. On the other hand, Vaughn et al., (1978) found poliovirus, echovirus, and coxsackie virus 150 feet from the contaminating source. Also, Hutchenson (1974) listed the distances that bacteria and viruses have traveled in ground water. Both coliforms and Clostridium welchii have been found approximately one kilometer from the contaminating source in limestone strata. Finally, Kenwick and

Gerba (1980) in their review presented data demonstrating that viruses had filtered approximately 45 feet through the soil and have moved 408 meters laterally through the ground water from the point source. They concluded that sandy and organic soils were poor virus adsorbers and that clay soils were good viral adsorbers. (Also, see Berg, 1974 for the mechanism of viral adsorption). They noted that viral survival in ground water previously has been recorded at 28 days, Wellings et al., (1975).

The fifth and final evidence includes the survival and lateral travel of microorganisms in ground water. Butler et al., (1954) noted that pollution travel in waste water followed two directions: (1) percolation of the bacteria and chemicals downward toward the ground water flow; and (2) the movement of bacteria and chemicals with the ground water flow. They found that pollution may not be stopped by the filtration of waste water through five feet of soil, especially in sites containing coarse soils, however, pollution was decreased with the use of finer soil. When the ground water is near the surface, bacteria will enter it through porous soil, root channels, or rodent holes. The investigators found no connection between infiltration rate and the removal of bacteria. The organic mat at the surface of the soil removed a large number of bacteria associated with the suspended solids. They believed

that bacteria build-up in the soil was due to the long survival time of E. coli. Finally, they found that the lateral transfer of chemicals was faster than that of bacteria. Chemicals were reported to travel varying from a few feet to several miles and from 2 to 30 times as far as the coliforms when both were introduced at the same time.

The significance of microorganisms from leachates draining into the ground water and moving laterally is that they cause diseases to persons drinking contaminated water. This fact comes from the many reports on this subject written by McCabe and Craun (Craun and McCabe, 1973; McCabe and Craun, 1975, Craun et al. 1976: and Craun. 1979). Specifically Craun and McCabe (1973) stated that ground water use by people contributed to 30,594 of cases due to bacterial, viral, parasitical and chemical contamination during the years 1946 to 1970. In all, 57% of the water born outbreaks in the United States and 49% of the cases were due to people drinking untreated ground water from private sources. This was a result of poor construction and poor location of wells. Examples of these microorganisms usually found in ground water are those associated with fecal material. Roebeck (1969) listed the following diseases usually transmitted by ground water: cholera, salmonellosis (Salmonella typhimurium) and infectious hepatitis. Furthermore, poliovirus, echovirus, and coxsackievirus have been found in

ground water beneath land treatment sites (Keswick and Gerba, 1980).

As about 20% of the population in the United States uses ground water for drinking purposes and the percentage (50%) of infections due to ground water use is high, the conclusion must be made that microorganisms travel in contaminated water (Keswick and Gerba, 1980). Thus, bacteria in leachate escaping from landfills can survive and move horizontally and vertically to contribute to the ground and surface water pollution.

CHAPTER III

LANDFILLS, LYSIMETERS AND OTHER EQUIPMENT USED

A. DESCRIPTION OF LANDFILLS AND LYSIMETERS

The principal materials assayed in this investigation were the leachate and solid waste obtained from landfills and lysimeters in the Cincinnati area. These sampling sites included the commercial landfill, the five Boone County landfills and lysimeters, nineteen Center Hill lysimeters and six lysimeters at the University of Cincinnati. Their locations are shown on a map in Figure 2, and a description of these sites is given in Table 6.

1. Commercial Landfill

The commercial site used in this study was possibly one of the five largest landfills in the United States; it covered some 600 acres of suburban land and was located north of Cincinnati. During the period of this research the landfill received 2500 tons of waste daily from Indiana, Kentucky, and Ohio. This was dry solid waste, and originated from municipal, hospital, commercial, and industrial sources (Commercial Landfill Operators, 1981, personal communication). See Figure 3.

This landfill was started in 1945 as an open dump. During the 1960's large volumes of leachate collected pro-



FIGURE 2. LANDFILLS AND FIELD AND LABORATORY
LYSIMETER SITES

TABLE 6. LANDFILL SAMPLING SITES

Landfill	Sampling Site Designations	Dimensions and Placement Feet (m)	Solid Waste Added ^a		Date of Landfill, (Lysimeter) Prepared	Date of Last Waste Deposit
			Type	Amount tons (metric tons)		
Commercial Full-Scale, Operational	CL-1 through CL-5	600 Acres in Soil	Municipal, Hospital, Commercial	Millions of Tons	1954	Daily (Active)
Boone County, Kentucky	TC-1	36 x 149 x 8.5 (9.1) x (45.4) (2.6) in Soil	Municipal	435 (395)	1971	June 1971 (Inactive)
Boone County, Kentucky	TC-2A through TC-2C	12 x 6 (3.7) x (1.8) Steel Cylinders in Soil	Municipal	3 (2.7)	1972	August 1972 (Inactive)
Boone County, Kentucky	TC-2D	28 x 28 x 10.5 (8.5) x (8.5) x (3.2) in Soil	Municipal	117 (106)	1972	August 1972 (Inactive)
Center Hill, Cincinnati, Ohio	CH-1 through CH-19	12 x 6 (3.7) x (1.8) Steel Cylinders ^b	Municipal Sewage Sludge, Industrial	3 (2.7)	1974 1975	Nov. 1974 ^a April 1975 (Inactive)
Univ. of Cincinnati Cincinnati, Ohio	UC-A through UC-F	2 x 2 (0.6) x (0.6) in Steel 55 gallon Drums at 20 C	Municipal Sewage Sludge, Hospital	150 lbs (68 kg)	1976	August 1976 (Inactive)

a Wet weight

b CH-1 through CH-15 were in the soil; CH-16 through CH-19 were in an unheated building.

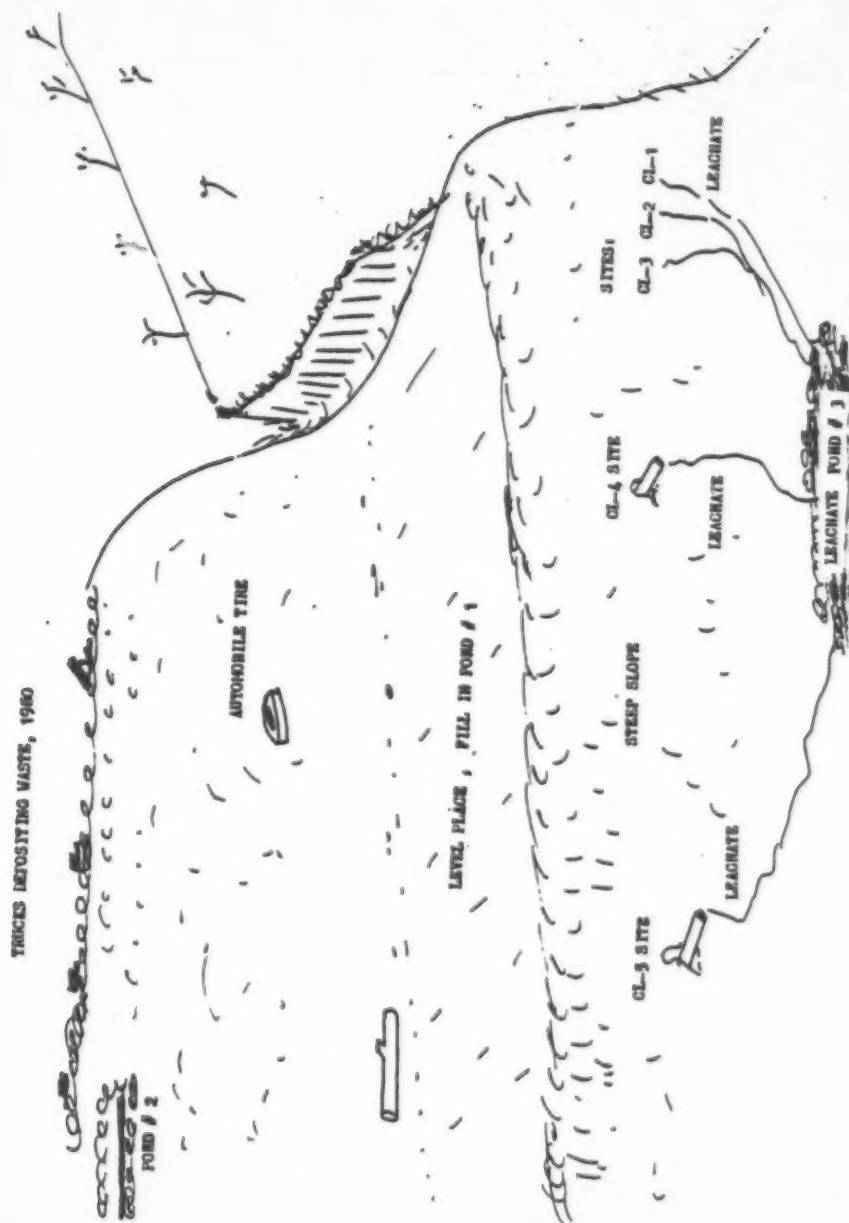


FIGURE 3. A SKETCH OF THE COMMERCIAL LANDFILL SHOWING THE FIVE SAMPLING SITES

ducing a 15 acre pond. The pond, #1, was drained in 1970 by pumping the leachate to the upper pond #2 where it was deodorized with ozone and then pumped into the Hamilton County Sewer System (Brunner, 1980, personal communication). Following this in 1970, the landfill operators filled-in the pond area, pond #1, with municipal and industrial waste, and placed two horizontal pipes (referred to as CL-4 and CL-5) which drained the remaining leachate from the landfill to a holding pond, #3 (Commercial Landfill Operators, 1980, personal communication). Daily waste was placed on top of the landfill about 150 ft from the base. On a level with CL-4 and CL-5 but to the North edge of the landfill, directly above the pond #3, leachate flowed from the soil of three sites, CL-1, CL-2, and CL-3. The leachate from these sites flowed into pond #3. Leachate samples from all five sites (CL-1 through CL-5) were used in this research.

2. Boone County Landfills and Lysimeters

The Boone County landfills and lysimeters were constructed and operated by the Solid and Hazardous Waste, Research, and Development, USEPA for about nine years. These landfills and lysimeters were built for experimental purposes to determine the long term properties of municipal landfills, such as the possible environmental damage brought about by the evolving methane gases or leachate draining

from these landfills. These landfills and lysimeters were placed on a ten acre portion of land leased by the USEPA from Browning-Ferris, Inc. who operated a sanitary landfill five miles west of Walton, Kentucky in Boone County. Their site covered 237 acres. This municipal sanitary landfill company serviced Boone and Kenton Counties. Thus, all municipal solid waste used at the Boone County experimental site came from these counties. After nine years the operation of this site was terminated due to reallocation of resources in response to the hazardous waste problem and the attainment of a quasi-steady state condition of the leaching and waste degradation processes occurring within the landfill. The site contained two experimental test cell experiments (i.e. TC-1 and TC-2 A, B, C, and D). These test cells were closed by September 30, 1980.

TC-1, the largest and the most frequently studied unit during this research, was constructed in 1971. (See Figure 4 for a picture of the site under construction). The dimensions are listed in Tables 6 and 7 and Figure 5. TC-1 was a trench type landfill that was 149 feet (45.4 m) long by 30 feet (9.1 m). The bottom sloped from 8 1/2 feet (2.59 m) below ground level to 0 feet (0 m) at each end. This bottom was covered with a clay liner placed on top of a 30 mil (25.4 μ m) chlorosulfonated polyethylene plastic liner, which caused the leachate to drain down into plastic

Reproduced from
best available copy.

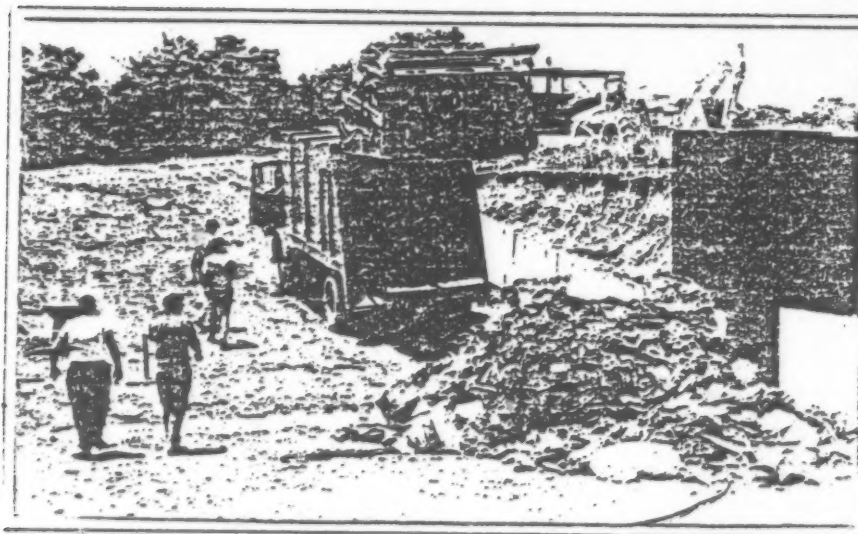


FIGURE 4. THE CONSTRUCTION OF THE BOONE COUNTY
LANDFILL IN 1971

TABLE 7. Boone County Lysimeters^a and Landfills^a

Landfill or Lysimeter Designation	Cell Enclosed In	Cell Size Meters (Feet)	Weight Kilograms (Pounds)	Density Kg/m ³	Initial Moisture %
TC-1 ^b	Soil	45.4 length, by 9.2 width, by 2.59-0 depth. (149x30x8.5-0)	395,454 (871,827)	592	29.9
TC-2A ^c	Steel cylinder	3.66 length by 1.83 diameter (12 x 6)	2,639 (5,817)	392	22.5
TC-2B ^c	Steel cylinder	3.66 length by 1.83 diameter (12 x 6)	2,898 (6,389)	431	27.1
TC-2C ^c	Steel cylinder	3.66 length by 1.83 diameter (12 x 6)	2,813 (6,202)	418	24.1
TC-2D ^c	Soil	8.53 square; 2.4 height (28 x 28 x 8.0)	106,200 (234,128)	598	31.8

a - All landfills and lysimeters were filled with municipal solid waste.

b - Built in June 1971.

c - Built in August 1972.

d - Wet weight.

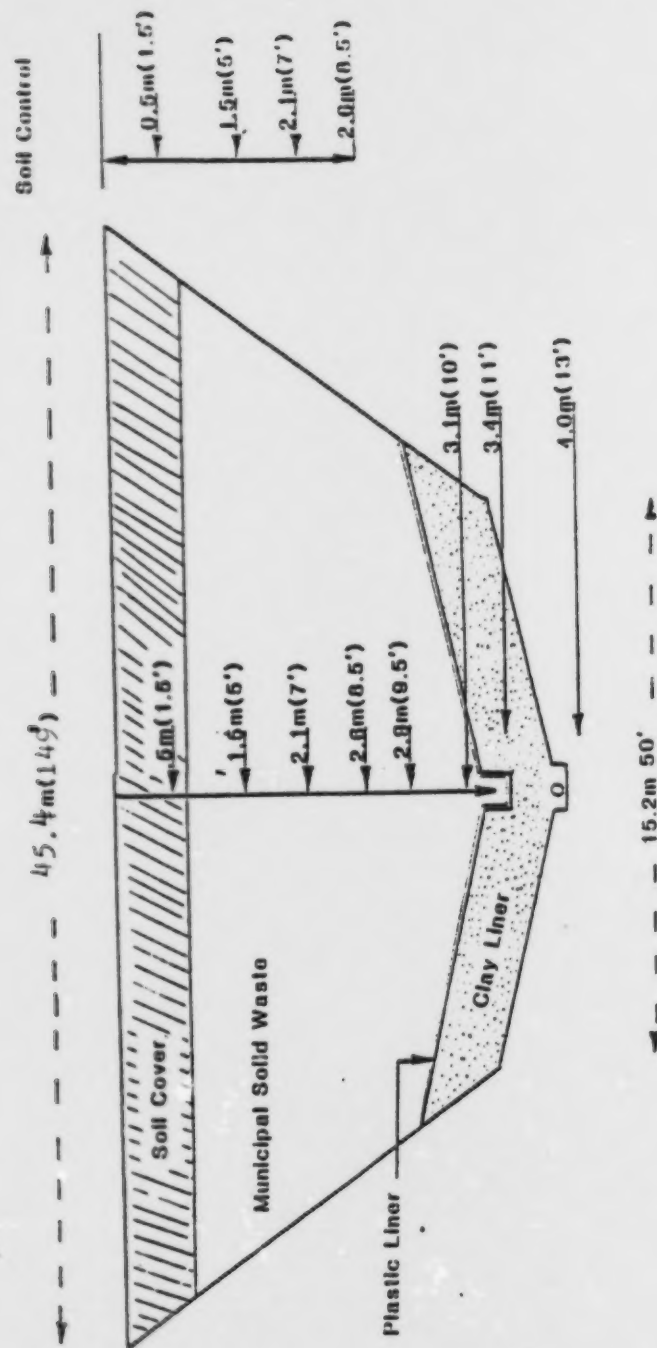


FIGURE 5. DESIGN OF TEST CELL 1 OF THE BOONE COUNTY LANDFILL

pipes at the bottom of the landfill. These pipes extended from this central pipe to the side of the landfill where sampling ports were positioned. Leachate obtained in this manner and from these pipes was called TC-1U, which drained the upper liner, the clay. The lower pipe TC-1L, drained the polymeric liner, but leachate from this source was not used in this study. One measurement of the fecal material placed in this landfill along with the municipal waste was the disposable diaper capacity; this was determined to be 0.7% of the dry weight.

Also on the site was TC-2, a group of four test cells constructed in 1972, which were labeled TC-2A, TC-2B, TC-2C and TC-2D. The first three were contained in large scale steel lysimeters [12 ft. (3.66 m) tall and 6 ft. (1.83 m) in diameter]. They were placed in the ground 9 ft. (2.74 m) below the soil surface on a concrete pad with their top surface exposed to the air. They contained about three tons of compacted municipal waste. TC-2D, also a landfill in the soil, 28 x 28 x 8 ft. (8.53 x 8.53 x 2.4 m) which contained compacted municipal waste refuse. This cell was lined with a 30 mil (25.4 m) chlorosulfonated polyethylene plastic liner covering the floor and four walls. A polymeric top was placed on top of the cell according to a prearranged schedule to limit the amount of rainwater infiltration to the cell. the leachate from these four cells was

obtained from pipes extending from each cell to a sampling center.

3. Center Hill Lysimeters

The purpose of the Center Hill experiment was to determine the effect of co-disposal of industrial sludges on municipal solid waste and to determine the possibility of environmental insult by such a practice. In addition, studies were made to determine the effects of varying water infiltration, landfills containing moisture-saturated waste, pH modification of landfills, co-disposal with sewage sludge, and finally the effect of temperature variation on leachate and gas production during the solid waste decompositions (Jackson and Streng, 1976).

For these studies nineteen lysimeters were assembled at the U. S. Environmental Protection Agency facility at Center Hill, Cincinnati, Ohio. In November 1974 thirteen lysimeters were constructed. The following April, numbers 9 and 10 were constructed along with numbers 16 through 19. All of these lysimeters were shaped as large steel cylinders 3.7 by 1.8 m (12 feet x 6 feet) and were coated with coal tar on the interior to prevent any interaction of the waste with the iron in the cylinder walls. Some of these lysimeters were closed at the top. See Figure 6 for the basic design. Lysimeters 3, 4, and 16 through 19 were sealed

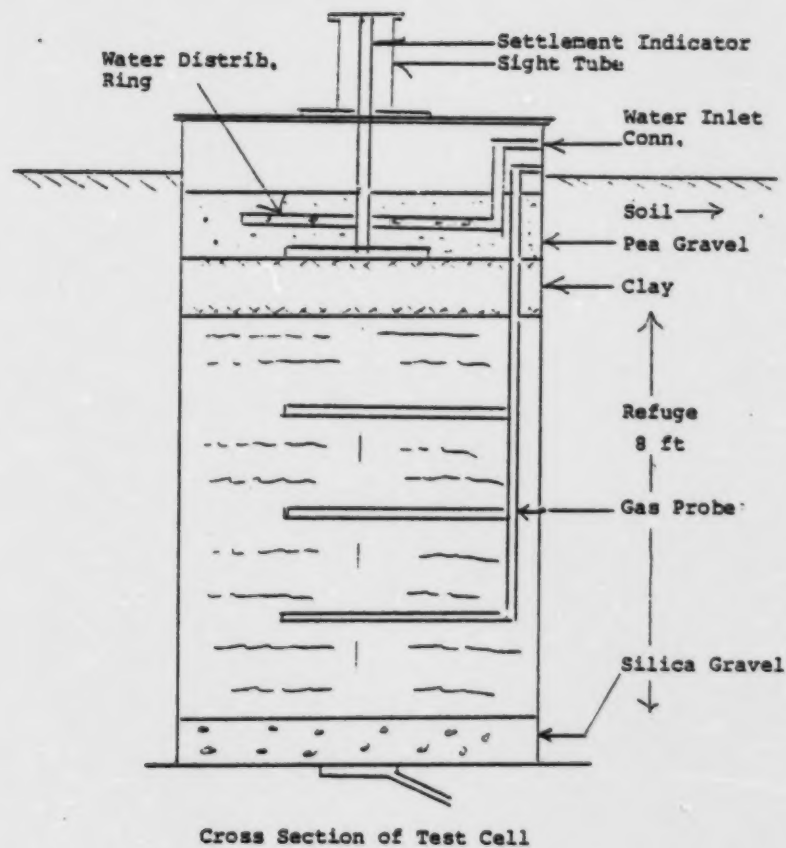


Figure 6. Design of the Center Hill Lysimeters.
(from Jackson and Streng, 1976)

permanently. Lysimeters 9 and 10, and 12 through 14 were left open, permanently. The rest were open and closed as designated by a schedule. The 15 out-of-door lysimeters (Numbers 1 - 15) were placed on concrete blocks 3 m down in the ground which were covered by the soil to within 0.3 m of the top of the lysimeter. A fiber glass roof was constructed over these 15 lysimeters to prevent the sun from raising their temperatures. Also, lysimeters 16 through 19 were housed inside a high bay area to restrict their exposure to the extreme winter and summer temperatures.

The composition of these lysimeters and some of their parameters are listed in Table 8. Lysimeters 1 through 4 were controls which were used to test the effect of various amounts of water additions on municipal solid waste stabilization. Lysimeters 5, 6, and 7 were used to test the effect of sewage sludge on municipal solid waste. Lysimeter number 8 received CaCO_3 which served to neutralize the low pH produced by the fermentation of the microorganisms. Lysimeters 9, 10, 12 through 14, and 17 were used to test the effect of acid sludges on stabilization. Lysimeter number 15 contained packets of Sabin attenuated poliovirus placed in the municipal solid waste, which were removed periodically to determine the poliovirus survival in landfills. The last four lysimeters, numbers 16 through 19, were housed indoors to determine this temperature restriction on CH_4 (methane)

TABLE 8. CONTENTS OF CENTER HILL LYSIMETERS^a

Cell No.	Solid Waste Total Weight In kg (lbs)	Additions to Municipal Solid Waste in Each Lysimeter (weight in Kilograms)	Total Water Added/Year In mm (inches)
1	3025 (6668)	None	203 (8)
2	2989 (6590)	None	406 (16)
3	3007 (6630)	None	610 (24)
4	3002 (6618)	None	813 (32)
5	3001 (6616)	Sewage Sludge (68)	406 (16)
6	2919 (6435)	Sewage Sludge (204)	406 (16)
7	2964 (6535)	Sewage Sludge (680)	406 (16)
8	2994 (6601)	CaCO ₃ (90)	406 (16)
9	3001 (6617)	Petroleum Sludge (1518)	406 (16)
10	2998 (6610)	Battery Production Waste (1291)	406 (16)
11	2924 (6446)	Saturated with H ₂ O (1294)	406 (16)
12	3048 (6720)	Electroplating Wastes (1191)	406 (16)
13	3006 (6626)	Inorganic Pigment Waste (1420)	406 (16)
14	3015 (6646)	Chlorine Production Brine Sludge (2039)	406 (16)
15	3010 (6636)	Polio Virus	406 (16)
16	2996 (6604)	None	406 (16)
17	2998 (6610)	Solvent Based Paint Sludge (1604)	406 (16)
18	3000 (6613)	None	406 (16)
19	3012 (6640)	None	406 (16)

gas introduction and waste stabilization. References to this work include Jackson and Streng (1976), Streng (1977), Walsh and Kinman (1979), and Walsh, et al., (1981).

4. University of Cincinnati Lysimeters

a. Purpose

Six lysimeters were built at the University of Cincinnati for the purpose of determining the survival of pathogenic microorganisms in landfills containing mixtures of sewage sludge (SS), municipal solid waste (MSW), and hospital waste (HW). These laboratory lysimeters were constructed on August 16, 1978. See Table 9. Lysimeters UC-B and UC-E were the test lysimeters and the rest were controls. Lysimeter UC-B contained mixtures of SS and MSW, while UC-E contained a mixture of HW and MSW. Two of the controls contained MSW (UC-C and UC-D), while one lysimeter contained SS (UC-A) and the other HW (UC-F). All lysimeters were incubated at 20 C. Microbial tests to determine the survival of fecal indicators and the pathogenic microorganisms in the leachate were done at periodic intervals during a period of two to three years. Afterwards, these lysimeters were opened up and the contents were examined for microbial presence.

TABLE 9. DESCRIPTION AND PURPOSE OF LABORATORY LYSIMETERS

Lysimeters	Contents ^{a,b}	Purpose
UC-A	Sewage Sludge	Microbial control for the sewage sludge used in lysimeter UC-B.
UC-B	Municipal Solid Waste Plus Sewage Sludge	To determine the impact of municipal sewage sludge additions on the rates of decomposition and formation of gas and leachate. Also, to elucidate microbial changes in the leachate.
UC-C	Municipal Solid Waste	Control on microbial numbers and species.
UC-D	Municipal Solid Waste	Same as lysimeter UC-C.
UC-E	Municipal Solid Waste Plus Hospital Waste	To determine the impact of pathogens on municipal solid waste and pathogen ability to survive landfill conditions.
UC-F	Hospital Waste	Microbial control on the hospital waste used in lysimeter UC-E.

a = 208.2 liters in each 55 gallon drum.

b = Lysimeters maintained at 20 C in a constant temperature room.

b. Lysimeter Design

The six lysimeters at the University of Cincinnati were designed to:

1. hold 200 to 300 lbs. (91 to 136 K) of solid waste
2. withstand the compaction forces reaching a waste density of 800 lbs/yd³ (474 kg /m³)
3. be air tight for the collection of gases, and to provide anaerobic conditions and
4. to have a liquid flow system such that added water would flow evenly over the top surface of the waste, would filter down evenly through the waste, and finally would be stored for a period of time in the base of the lysimeter before sampling.

The final design is shown in Figure 7.

These six lysimeters were designed and constructed by Dirk Brunner, of the USEPA, Dr. P.V. Scarpino and Jean Donnelly of the University of Cincinnati, Dave Streng and Dan McCabe of Environmental Enterprises, Inc, Cincinnati, Ohio.

All lysimeters were made by adapting 55 gallon drums to fulfill the purposes described above. These steel lined

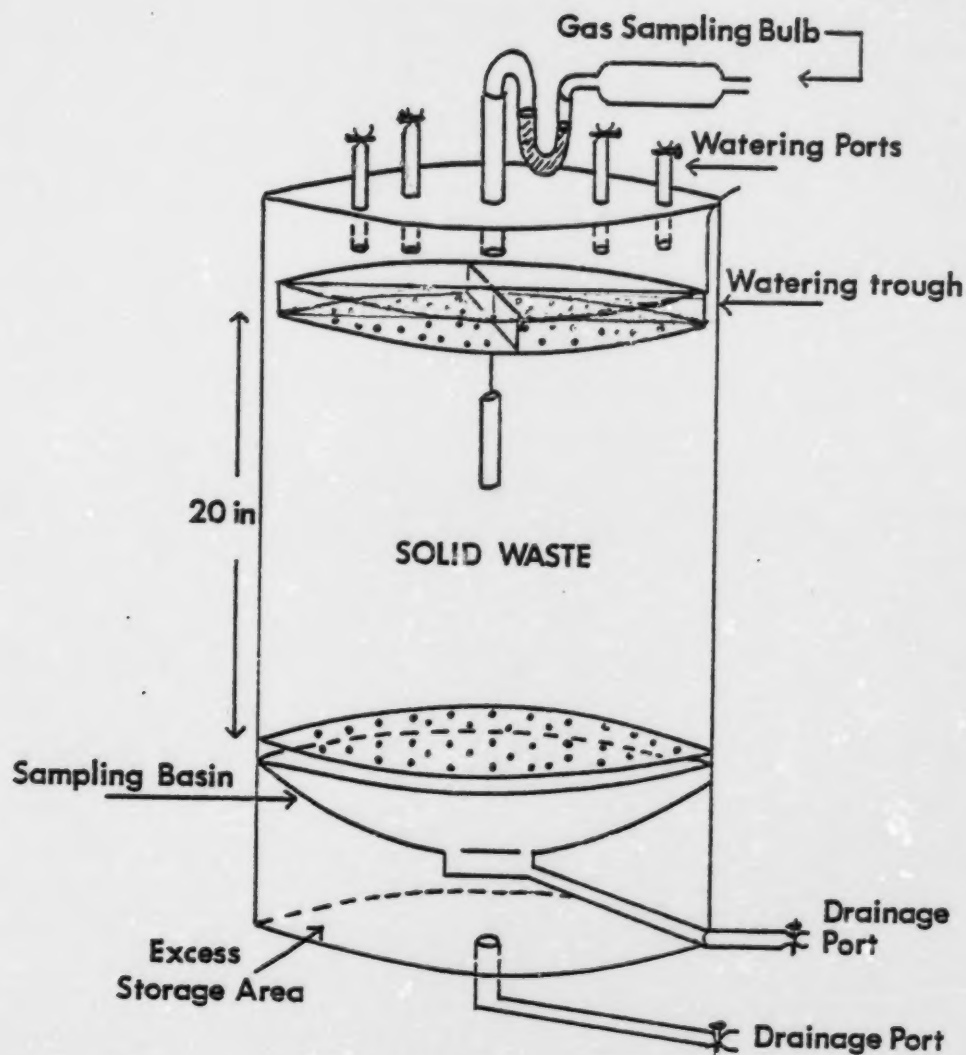


FIGURE 7. SCHEMATIC OF EXPERIMENTAL LABORATORY
LYSIMETER

drums were steamed clean and were coated with epoxy to prevent possible interaction of the leachate with the iron in the lysimeters. The lids on each lysimeter had six openings as shown in Figure 7. Five of the openings contained pipes extending through the lid. The first pipe in the center of the lid was used for attaching gas sampling bulbs and gas bags. The second, third, fourth, and fifth pipes had glass manometers attached to them by rubber hoses. These small glass manometers, 15 cm high, contained water which prevented oxygen (from the air) from entering the lysimeters and the release of gases due to increased gas pressure within the lysimeters. In addition, the weekly water ("rain") was added to the lysimeter through these manometers. When not in use for watering purposes, sealed rubber hoses were attached to the open ends of the manometers to decrease the water evaporation and the absorption of oxygen in the manometer water. Finally, the sixth opening (1/4 in or 0.64 cm in size) was used to pass the thermister wire leading from down inside the middle of the waste to a readout on the outside of the lysimeter. This opening was sealed to prevent the gases produced by the waste within the lysimeter from escaping to the outside.

Attached to each lysimeter lid were constructed compartments made of fiberglass (PVC) designed to provide for the even flow of water through the waste from the top to

the bottom. The first of these compartments was a watering trough, 28 in in diameter (71 cm) which was attached to the under-surface of the lid. This watering trough with 1/4 in (0.64 cm) holes in the bottom contained a fiber glass cross section which divided the interior space into four equal parts (quadrants). (See Figure 7.) This unit was designed to insure that the weekly water additions would fall only into the designated four chambers from each of four manometer openings directly above, and would not spill into another quadrant. This would thus insure an equal distribution of the water draining over the waste in case the lysimeters were not level. In addition, plastic rings (3 in or 7.62 cm in diameter and 1/4 in or 0.64 cm high) were attached to the bottom of the watering pan to insure that the water draining through the pan would drip directly below, and would prevent water from running to one side or the other, should the lysimeters not be level. The next unit below the watering trough was the solid waste reservoir. Here, some 100-300 lb (45-136 kg) of waste was compacted to approximately 800 lbs/yd³ (474 kg/m³). The compacted waste rested upon a fiber glass screen strong enough to withstand the compaction forces necessary to achieve this density. Below the screen was a funnel which guided the leachate to a small container. This container, below the funnel, held about 300 ml of leachate. At one side was a

hole to allow for leachate overflow into the larger leachate storage area directly below. From this leachate storage area and also from the small container, two plastic tubings carried the leachate directly to the outside.

c. Solid Waste Added to the Lysimeters

1.) Sewage sludge -- The sewage sludge was obtained from the Metropolitan Sewer District, Hill Creek Treatment Plant, Cincinnati, Ohio, 45204, twenty-four hours before the lysimeters were constructed. The sewage sludge coming off the vacuum filter on August 15, 1978, was a mixture of the freshly settled primary sludge and the previous day's sludge which had been settled with the aid of a polymer. The sludge was taken from a moving belt just after it had dropped from the filter. Following its collection, the sludge was added to the lysimeter which was then placed in the 20 C incubator.

2.) Hospital waste -- On August 16, 1978 one ton of hospital solid waste was obtained from the Cincinnati General Hospital, brought directly from patients' rooms. The waste was packed in plastic bags and was taken to the Center Hill facility. There it was shredded, using a Model F21-P shredder (W. W. Grinder Corporation of Wichita, Kansas), to sizes of two to six inches in length. This shredded waste was then placed in plastic bags until it was

packed in the lysimeters. The purpose of shredding the waste was to insure proper mixing, and to aid in the compaction process.

3.) Municipal Waste -- The municipal waste was collected from the residential areas of Columbus, Ohio and was taken to the City of Columbus, Sanitation Division, Pulverizer Plant, Columbus, Ohio, 43207. There it was shredded to sizes of two to six inches in length. It was then well covered and placed in a truck and driven to the Center Hill Facility in Cincinnati, Ohio. At this time it was weighed and placed in plastic bags in preparation for its deposition into designated lysimeters.

Prior to the construction of the lysimeters, calculations were made of the ratios of sewage sludge (SS) to municipal solid waste (MSW), and hospital waste (HW) to MSW occurring in the Cincinnati area. These ratios of waste, which would contribute a maximum concentration of pathogens to the landfill, were selected for use in the test lysimeters. Furthermore, the calculations were based on the waste concentration expected from a community the size of Cincinnati with a population of 500,000.

4.) Sewage sludge / municipal solid waste mixture (SS/MSW) -- The following concentrations were used in the calculations of the SS/MSW ratio:

1. Municipal solids dry weight (dw) (Fair, Geyer and Okum, 1964), (dw) = 0.119 lbs cap/day.
2. Solid content of sewage sludge = 29% The sludge was taken on 7/9/78 from the vacuum filter of the Mill Creek Treatment Plant.
3. A city the size of Cincinnati produced 800 tons (estimated) of refuse a day, (wet weight) (Brunner, 1980 personal communication), (1 ton = 2,000 lbs.).

Calculations: $\frac{(\text{Refuse/Day}) (\text{lbs/ton})}{\text{population}} = \text{lbs/cap/day}$

$$\frac{800 \times 2,000}{500,000} = 3.2 \text{ lbs/cap/day refuse in Cincinnati}$$

$$\frac{\text{Solids Produced (dw)}}{\text{Fraction of Solids in Sludge}} = \text{Sludge Produced (ww)}$$

$$\frac{0.119}{0.29} = 0.41 \text{ lbs/cap/day SS (ww) produced}$$

The calculation of the (SS:MSW) ratio is as follows:

$$\frac{\text{Refuse lbs/cap/day(ww)}}{\text{Sewage sludge lbs/cap/day (ww)}}$$

$$\frac{3.2}{0.41} = 7.8 \text{ Ratio of MSW (ww) to SS (8:1).}$$

However, a ratio of 1:4 instead of 1:8 was chosen, so as to increase the amount of sewage sludge in the lysimeters in order to cover any unusually large deposits of sewage sludge in the landfills. For example, the sewage sludge is probably never dumped into landfills by communities on a

regular basis, but is deposited in large batches at irregular intervals. Therefore, to account for higher concentrations of SS at various areas in landfills, it was decided to increase the percentage of the sewage sludge from 12.4% (1:8 as calculated above) to 20 or 30%. This would represent a possible maximum concentration of the sludge occurring in landfills.

5.) Hospital waste/Municipal solid waste mixture (HW/MSW) -- The following data used in the HW/MSW calculations came from Brunner (1977 - personal communication) and included the following:

There are approximately 9,000 hospital beds in Cincinnati. Each patient generates 10 lbs of refuse/day. A city the size of Cincinnati produces 3.2 lbs/cap/day municipal refuse.

The calculation:

Hospital bed x lbs/day waste = Hospital refuse/day

9,000 x 10 = 90,000 lbs hospital refuse/day

$\frac{\text{lbs refuse/day}}{\text{population}} = \text{ratio lbs/cap/day}$

$\frac{90,000 \text{ lbs refuse (hospital waste)/day}}{500,000} = 0.18 \text{ lbs/cap/day}$

$\frac{\text{MSW/cap/day}}{\text{HW/cap/day}} = \text{Ratio MSW:HW}$

$\frac{3.2 \text{ MSW lbs/cap/day}}{0.18 \text{ HW lbs/cap/day}} = 18. \text{ The ratio of MSW to HW (18:1)}$

Thus, using a ratio of 18:1, 94.5% of the waste should be MSW and 5.5% should be HW. Again, to use possible maximum concentrations, 20 to 30% of the waste in the lysimeter was chosen to be the HW, because the hospital waste is dumped in batches and is not distributed on an even basis day by day.

6.) Components of the Solid Waste -- The contents of the municipal solid waste (MSW) and hospital waste (HW) used in the lysimeters are listed in Table 10. This list was prepared by examining dried waste following the moisture determinations. For these moisture determinations, all waste was heated at 100 C for two weeks. These items from the municipal waste and the hospital waste were then classified according to the eight categories listed by Neissen and Chansky (1970) and compared with national refuse averages. These comparisons are shown in Table 11. Although the MSW and HW are listed in terms of dry weight, all of the items, except for the food contained only low amounts of moisture and were thus comparable to the national averages. In general, the concentrations of the eight components contained in the MSW and HW were smaller when compared to those of the national averages except for the food (which was absent), the high percentage of papers, and the lack of wood present in hospital waste.

TABLE 10. CONTENTS OF THE MUNICIPAL SOLID AND HOSPITAL SOLID WASTES

Type of Waste	List of Waste Materials ^a
Municipal Solid Waste	Wood, metal top of pop bottle, leaves, tin can, aluminum foil, plastic, newspaper, glass, twigs, popcorn, plastic tubing, foam rubber, plastic bags, hamburger wrapper, shoe sole, wire, cardboard, small metal pieces, rags, sock, plastic milk carton.
Hospital Waste ^b	Surgeon's gloves, bandages, sanitary napkin, packages of soap, gauze, paper towel, cracker wrapper, antibiotic tubes, newspapers, paper bandages, packing material, cellophane, plastic tubing, baby bottle nipple, blood transfusion equipment, rubber tubing, gauze and surgeon's gloves containing blood, cigarette butts, baby diapers.

a = As determined by examining dried samples.

b = The material was not clean, but was bloody or soiled in some manner.

TABLE 11. COMPOSITION OF THE DRIED^a MUNICIPAL AND HOSPITAL SOLID WASTES
USED FOR CONSTRUCTING THE UNIVERSITY OF CINCINNATI LABORATORY LYSIMETERS

List of Waste Components	Annual ^b Average (Wet Waste)	Percent Waste Municipal Solid Waste (Dry Waste)	Hospital Solid Waste (Dry Waste)
Glass	9.9	1.0	1.6
Metal	10.2	1.0	1.5
Paper	51.6	84.5	86.3
Plastics	1.4	5.6	5.3
Leather, Rubber	1.9	1.3	1.0
Textile	2.7	1.6	4.3
Wood	3.0	4.0	0
Food	19.3	1.0	0
Total	100.0	100.0	100.0
Moisture Content	-- ^c	36.6 ^d	24.9 ^d

a - The wastes examined were those samples used in the moisture determinations.

b - This is the "Estimated Annual Average National Refuse Composition" (on a yard-waste-free and miscellaneous basis) from Niessen and Chansky (1970).

c - Not applicable.

d - Percent of the total wet waste weight.

7.) Moisture Determinations of the Solid Waste --

The moisture determinations were made by wrapping waste in aluminum foil, placing them into 4 in. x 2 ft. x 5 ft. (0.1 x 0.6 x 1.5 m) steel containers with multiple one inch (2.56 cm diameter) holes. The steel containers were weighed before and after heating the waste at 100 to 105 C for three days and cooling for two days (Standard Methods, 1976). This was repeated until the weight remained constant. The moisture content averaged 70.0% for sewage sludge, 33.6% for the municipal solid waste, and 24.8% for the hospital waste.

8.) Size Determinations for Solid Waste Particles--

Size determinations were made of the municipal solid waste and the hospital waste. These dried wastes were sent to Thomas, Dean, and Hoskins (Great Falls, Montana 59405) who used sieve analysis. Their sieve unit consisted of a trommel with screens having square openings with inside dimensions of 1/2, 1, 2, 4, 6, and 8 inches (1.27, 2.54, 5.08, 10.16, 15.24 and 20.32 cm). The inlet end of the unit was raised 13 inches (0.33 cm) above the outlet end to allow the milled refuse to tumble through each screened area as the trommel was rotated. The material was then collected under each screened area and weighed. A portion of the original composite sample was dried at 100-105 C to determine the moisture content. The cumulative percent passing

each screen size was corrected for moisture content and then plotted. The plots of the municipal solid waste and the hospital solid wastes are shown in Figures 8 and 9. A particular percentage point on the graph indicated the percent of the waste particules that can pass through the particular screen size. For example, if the point in the graph was 30% at 4 inches (10.2 cm), then 30% of the particles will pass through the 4 inch screen. The results show that both graphs were similar and thus both wastes appeared to have particles of the same size. In both cases approximately 80 percent of the waste passed through the two-inch (5.0 cm) screen. This small size of the waste aided in the mixing and the compaction process. The purpose of the shredding was to aid in the mixing and in achieving a compaction density of at least 800 lbs/yd³ (474 Kg/cm³).

d. Lysimeter construction

1. Overview -- The main focus of the construction process was to quickly place all three waste types in the lysimeters in such a way as to damage as little as possible the microbes present in the waste. Namely, drying of the waste would kill the microorganisms, as would the elevated temperatures which occur when waste is oxidized in the presence of air. Thus, the plan was to obtain the sewage sludge on one day, put it in the lysimeter, and place the

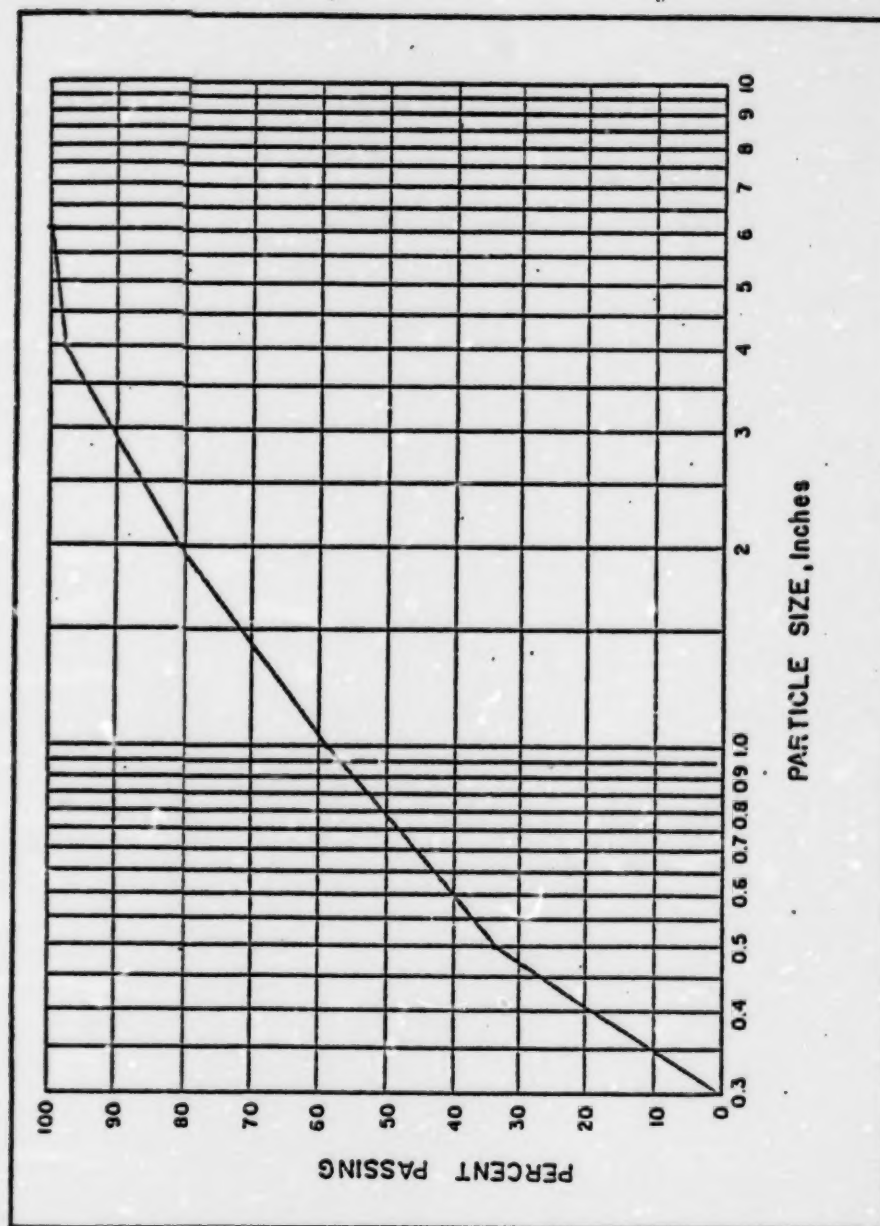


FIGURE 8. PARTICLE SIZES OF SHREDDED MUNICIPAL SOLID WASTE

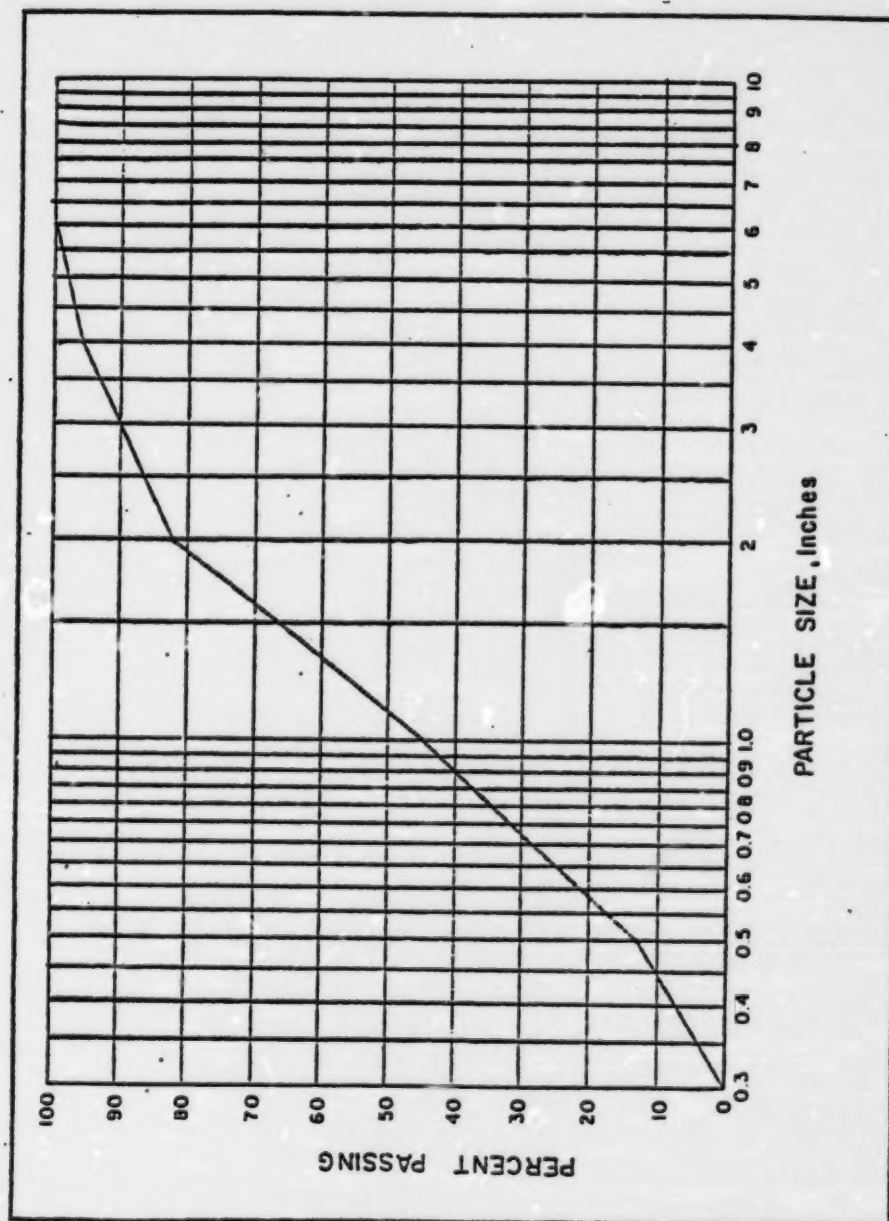


FIGURE 9. PARTICLE SIZES OF SHREDDED HOSPITAL WASTE

lysimeter in the 20 C incubator immediately. The next day the other wastes were picked up, shredded, and placed in the five lysimeters, then compacted, lids were attached, and these units were placed in the 20 C incubator. There, all lysimeter openings would be closed with plastic hoses and the initial water additions would be added at this time to cool the waste. The stages of construction described are as follows:

2. Securing wastes from sources -- On August 15, 1978, the sewage sludge (SS) was obtained from the treatment plant and placed in a weighed lysimeter, which was taken to the University of Cincinnati, weighed again to obtain the mass of the waste, and placed in the 20 C incubator. Additional SS for a second lysimeter was also obtained at this time and kept in the 20 C incubator until it was needed for the mixed waste lysimeter containing SS and municipal solid waste (MSW). On August 16, 1978, the other wastes, the MSW and the hospital solid waste (HSW) were obtained from their sources and brought to the Center Hill Facility.

3. Filling, compacting, and sealing lids of lysimeters -- The wastes were added to the lysimeters according to the experimental design listed in Table 9. All wastes were weighed, mixed, and bagged until they were used to fill the lysimeters. The solid waste was added in stages and compacted in between each stage. The type of waste and the

amounts placed in the lysimeters are also shown in Table 12, as are the moisture determinations. The average weight (ww) of waste added to each lysimeter was 165.1 lb (75 kg) and the average dry weight was 77.7 lbs (35.2 kg). The compaction density of each lysimeter UC-A through UC-F was 1541, 1144, 970, 1006, 847, 604 lbs/ yd³ (914, 678, 575, 596, 502, and 358 kg/m³). The density (weight/volume) of the solid waste was determined during the compaction process. During this process a thermister was inserted into the middle of the solid waste. More waste was packed around it to hold it in place. Next bentonite, mixed into a paste, was packed around the top of the packed waste to prevent the weekly additions of water from channeling down the sides between the waste and the wall of the lysimeters. Next, the lid was secured to the top of the lysimeter using Metal Sealant #2084, Scotch Brand (3M Company, St. Paul, Minn.). Then a metal belt was tightened to secure the lid to the lysimeter. Lastly, the thermister wire, leading from inside the lysimeter was sealed with Black Silicone Rubber Sealant #732-BL11 (Dow Corning Corporation, Midland, Michigan, 48640).

4. Placement of lysimeter in incubator, and initial temperature measurements -- All lysimeters were then placed in the 20 C incubator at the University of Cincinnati as soon as possible after construction to counteract the initial high temperature that usually is present in newly

TABLE 12. THE COMPOSITION OF THE SIX UNIVERSITY OF CINCINNATI LYSIMETERS^a

Lysi-Meter	Total Wet Weight	Weight lbs (Kg) Solid Waste Added to Lysimeters					
		Sewage Sludge ^b 70.0% Moisture ^b		Municipal Waste ^b 33.6% Moisture ^b		Hospital Waste ^b 24.8% Moisture ^b	
		Wet Wt (Dry Wt)	% of Total Wt	Wet Wt (Dry Wt)	% of Total Wt	Wet Wt (Dry Wt)	% of Total Wt
UC-A	258.0 (117.0)	258.0 (117.0) 77.4 (35.1)	100				
UC-B	188.3 (85.4)	51.0 (23.1) 15.3 (6.9)	27.0	137.1 (62.2) 91.1 (41.3)	72.9		
UC-C	159.5 (72.3)			159.5 (72.3) 105.9 (48.0)	100		
UC-D	160.0 (72.6)			160.0 (72.5) 106.2 (48.2)	100		
UC-E	125.3 (56.8)			98.7 (44.8) 65.5 (29.7)	78.9	26.4 (12.0) 19.8 (9.0)	21.1
UC-F	101.2 (45.9)					101.2 (45.9) 76.1 (34.5)	100

a = All Constructed on August 18, 1978.

b = Solid waste samples were dried for two weeks at 100 C.

deposited waste (Brunner, 1977 personal communication). This high temperature, perhaps, would kill large numbers of bacteria present in the waste. The temperature of the MSW and the HSW as they were being loaded into the lysimeters were 102 and 74 F, respectively. (See Table 13.) After the lysimeters were placed in the 20 C incubator, the temperatures were taken every 6 hours for a month. These temperatures are listed in Table 14. To help reduce the temperatures, the first of the weekly water additions used to attain field capacity were added within an hour or two after the lysimeter placement in the 20 C incubator. The waste was weighed and placed in plastic bags to prevent drying and oxidation as both kill microorganisms.

e. Lysimeter Maintenance

1. Temperature -- All six of the lysimeters were kept in the 20 C incubator for two years and four for three years. During this time, the temperature, checked daily, averaged 19.5 C and varied from 18.5 to 20.0 C yearly.

2. Estimates of water volumes needed to reach field capacity -- As the municipal solid waste (MSW) and the hospital waste (HW) contained only 34% and 25% moisture, respectively (See Table 13) it was necessary to add a great deal of water in order to obtain saturation conditions (field capacity) and initiate leachate flow.

TABLE 13. TEMPERATURES OF SOLID WASTES PRESENT IN THE LYSIMETERS
IMMEDIATELY AFTER COMPLETION

Lysimeter Designation	Composition	Temperature ^b	
		3:30 a.m.	7:30 p.m.
1 UC-A	Sewage Sludge (SS)	--	73 (22.7)
2 UC-B	MSW & SS	80 (26.6)	76.5 (24.7)
3 UC-C	MSW	101 (38.3)	97.5 (36.4)
4 UC-D	MSW	102 (38.9)	99.5 (37.5)
5 UC-E	MSW & HW	79 (26.1)	75.5 (24.2)
6 UC-F	HW	74 (23.3)	75.5 (24.2)

^a Lysimeters were filled on 8/17/78.

^b MSW upon arrival from Columbus was 79 F (26.1 C)

TABLE 14. INITIAL TEMPERATURES OF THE UNIVERSITY OF CINCINNATI LYSIMETERS
IN THE 20 C INCUBATOR (0 TIME TO 111 HOURS)

Hours at 20 C	UC-A	Temperatures Inside the Lysimeters (C)				UC-F
		UC-B	UC-C	UC-D	UC-E	
0 ^a	23.3	26.7	38.3	38.9	26.1	23.3
4	22.7	24.7	36.4	37.5	27.7	24.2
11	22.2	26.4	35.0	35.5	27.7	26.1
14	24.4	25.8	32.2	31.6	26.1	24.4
20 ^b	23.8	25.5	30.0	29.4	24.7	23.3
27	23.8	23.8	26.9	26.4	23.3	21.6
32	22.7	23.3	25.5	25.0	22.2	21.1
38	22.7	27.7	24.4	23.8	21.6	20.5
45 ^c	22.7	24.4	24.4	22.7	23.3	23.8
51	22.2	23.8	23.8	23.3	22.2	21.6
58	22.2	23.3	23.3	22.7	22.2	21.1
60	23.3	23.3	23.3	23.3	22.2	21.6
67 ^d	23.3	23.3	22.7	23.3	22.7	21.1
76	22.2	22.2	22.2	21.6	21.6	21.6
82	21.1	21.6	21.1	21.1	21.1	20.5
87	21.1	21.1	21.1	21.1	20.5	20.5
92 ^e	20.5	21.1	20.5	21.1	20.5	20.3
101	20.8	21.1	20.5	20.5	20.3	20.5
106	20.5	20.8	20.0	20.3	20.0	20.0
111	20.0	20.5	20.0	18.6	20.0	20.0

a, b, c, d, e = August 17 through the 21st, 1977.

The values for the initial water addition to each lysimeter came from estimates. Estimates were used because the moisture content of the municipal solid waste, hospital waste, and sewage sludge had not been determined at that time and was thus not available. The calculations used to estimate the water volumes needed per lysimeter were:

$$800 \text{ lbs/yd}^3 =$$

$$474 \text{ kg/m}^3 = \text{Density of the compacted waste (the desired density)}$$

$$0.2 \text{ yd}^3 = \text{estimate of waste volume}$$

$$800 \text{ lbs/yd}^3 \times 0.2 \text{ yd}^3 = 134 \text{ lbs refuse/lysimeter}$$

Assuming the refuse contained 25% moisture, then

$$134 \times 0.25 = 33.5 \text{ lbs of water in refuse}$$

$$134 \times 0.75 = 100 \text{ lbs of water needed to bring the waste to field capacity}$$

$$1.4 = \text{a moisture factor used to calculate field capacity (Brunner, 1977 personal communication)}$$

Therefore, we needed:

$$100 \text{ lbs} \times 1.4 = 140 \text{ lbs of water were required to add to each lysimeter to bring them to field capacity}$$

Assuming $2.2 \text{ lbs} = 1 \text{ kg}$, and $1 \text{ Kg} = 1 \text{ L}$ of water, then

$$\frac{140 \text{ lbs of water}}{2.2 \text{ lbs/kg}} = 63 \text{ L of water would be required.}$$

This then was the amount of water needed to bring the lysimeters to field capacity. For a six week period the water additions equal $63 \text{ L/wk}/6 \text{ wks} = 10.5 \text{ L of water/week/lysi-meter}$.

3. Leachate removal -- Leachate was removed on a weekly basis from the six lysimeters under a flow of nitrogen at their upper and their lower drainage ports. (See Figure 9.) The leachate for analysis was always removed from the fiberglass container hose. The leachate samples were then placed in a 5 C incubator and processed within 24 hours.

4. Weekly water additions--Water was added to each lysimeter to simulate 40 inches of rainfall in Cincinnati by adding enough water to produce 20 inches of leachate per lysimeter. Most landfills in this area produce 16 to 20 inches of leachate per year (Brunner, 1977, personal communication). In the first year the tap water for each lysimeter was placed in large 20 liter carboys in direct sunlight for one week to remove the chlorine. When algae appeared in the glass carboys during the first summer, this procedure was replaced by using cooled boiled tapwater (the cooling removed the chlorine). Before the weekly water additions were made to each lysimeter, the tap water was checked for the presence of chlorine using a DPD spot test (Standard Methods, 1976).

The volume of water added to each lysimeter was based on the following estimations: for the purpose of this experiment it was assumed that 40 to 50 inches of rain would fall in Cincinnati which would produce about 20 inches of leachate. Therefore, 20 inches of water were added to each lysimeter to produce 20 inches of leachate. Calculations of leachate produced/year in the lysimeters were:

$$\pi r^2 = \text{Surface area formula}$$

$$\pi \left(\frac{22.375}{2} \right)^2 = 393 \text{ in}^2 = \text{Surface area of lysimeters}$$

The calculated liters of leachate produced per year were:

$$393 \text{ in}^2 \times 20 \text{ inches} = 7860 \text{ in}^3, \text{ volume of water added yearly}$$

$$61 \text{ in}^3/\text{L} = \text{measurement of 1 liter}$$

$$\frac{7860 \text{ in}^3}{61 \text{ in}^3/\text{L}} = 128 \text{ L leachate}$$

Thus, the addition of 128 L of water to each lysimeter should produce 128 L of leachate. Accordingly, a plan schedule was devised for the monthly water additions. Each month was rated 1, 2, or 3 according to the amount of rain usually produced per month for the low, medium and high, respectively, rainy months which would produce low, medium, or high, respectively, volumes of leachate. Then, the number of months in each of the three categories was multiplied by the factors (1x, 2x, or 3x) to produce the number of water units per category (low, medium, or high) which when totaled

would give the total number of "rain fall units" per year. (See Table 15). In this case the total number of rainfall units was 23. Thus, when the total volume of water to be used yearly (128 L) was divided by the number of water units, the calculated volume of 5.6 L was determined. This then was the volume of water needed per lysimeter for the dryest months of the year.

$$\frac{128 \text{ L}}{23 \text{ units}} = 5.6 \text{ volume (Liters) of water in the low rain months.}$$

This number which when multiplied by 2X or 3X would give the volume of rain produced by the medium and high rainy months as shown in Table 16.

These volumes, 5.6, 11.2, or 16.8 liters of boiled, cooled tap water were added to the lysimeters during the low, medium, and high rainy months (Figure 10). Then these volumes were divided by the number of weeks per month and added on a weekly basis. Sterile graduates and aspirator bottles were used to add the water to each lysimeter to prevent the addition of any pathogens. Furthermore, to eliminate any oxygen transfer from the air to the lysimeter, the aspirator bottles and rubber tubing were flushed with nitrogen.

The comparative volumes of water additions and leachate removal are presented in Figure 11. During the first four weeks large amounts of water were added to bring the

TABLE 15. THE DETERMINATION OF RAINFALL UNITS/YEAR

Relative Amount of Rain per Month	Multiplication Factor	Number of Months	Extension
Heavy Rainfall			= 9
March, April, May	3X	3	
Medium Rainfall			= 10
February, June October November, December	2X	5	
Light Rainfall			= 4
July, August, September, January	1X	4	
Total Number of Units of Rainfall/ Year			23 Units

TABLE 16. THE TOTAL AMOUNT OF WATER ADDED/LYSIMETER/YEAR

Type of Months	Multiplication Factor	Number of Months	Volume of Water Added
Heavy Rainfall ^a	(3X 5.6) = 16.8	X 3	= 50.4
Medium Rainfall ^b	(2X 5.6) = 11.2	X 5	= 56.0
Light Rainfall ^c	(1X 5.6) = 5.6	X 4	= 22.4
			128.8 L

a = March, April, May
b = February, June, October, November, December
c = July, August, September, January

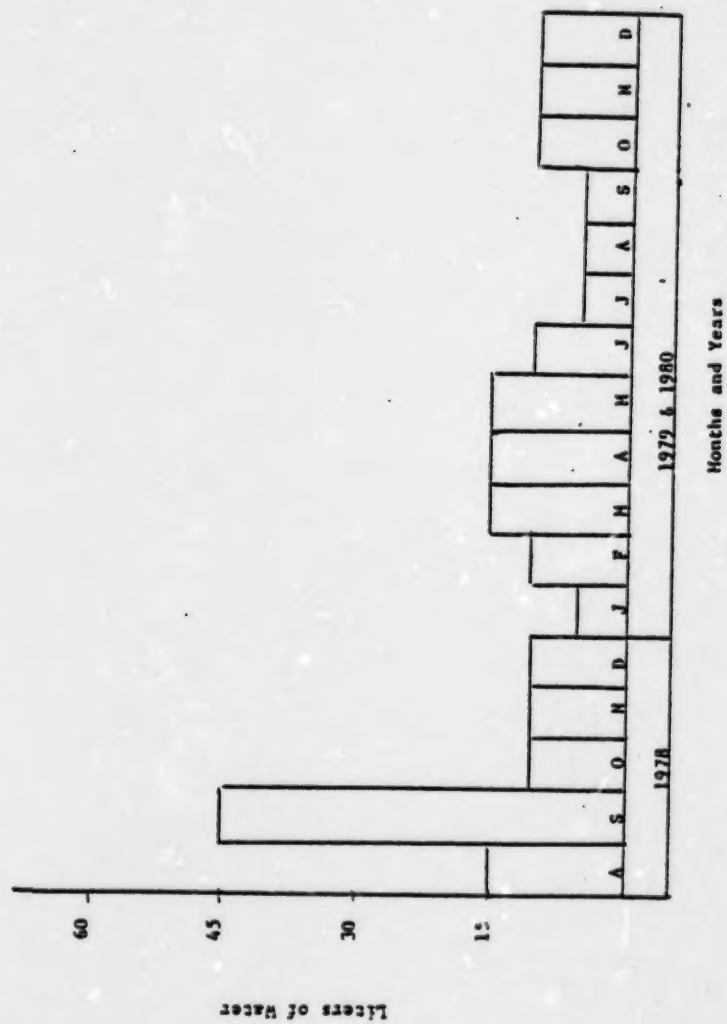


FIGURE 10. WATER REGIME FOR EXPERIMENTAL LANDFILLS

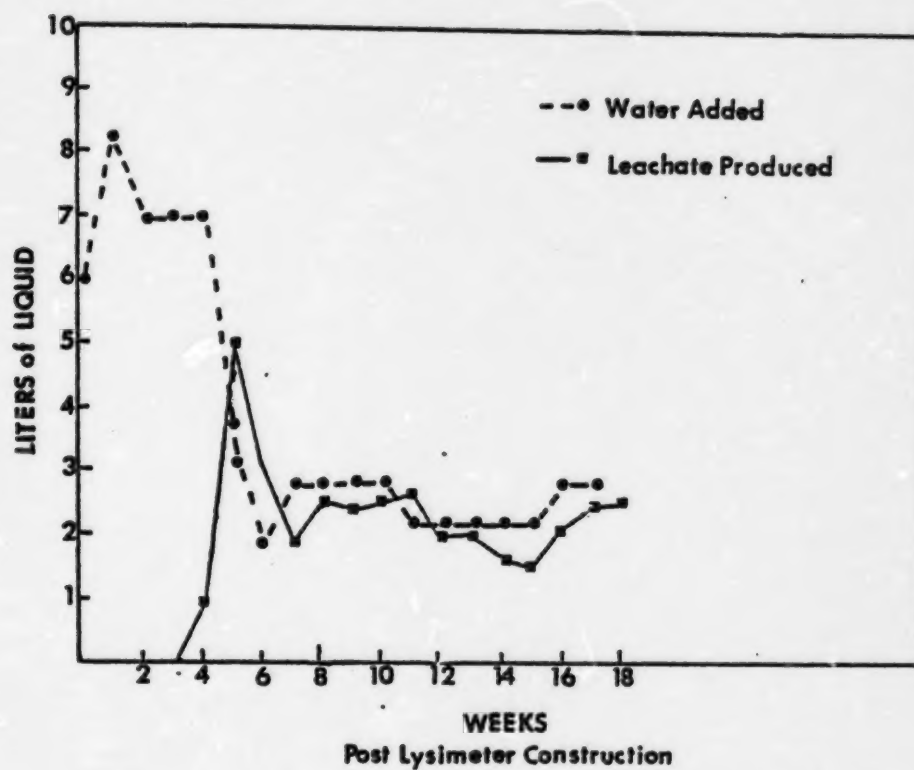


FIGURE 11. ADDITION OF WATER AND PRODUCTION OF LEACHATE IN THE LABORATORY LYSIMETER.

waste to field capacity. Field capacity was attained during the fourth week.

B. LABORATORY EQUIPMENT

1. Biological Safety Hood

A safety hood was used in the experiments for handling all mycobacteria and fungal species as they were considered potentially pathogenic. (Communicable Disease Center) Classification of Etiologic Agents on the Basis of Hazard, (1976). In addition, the fungi (whether pathogenic or not) may cause allergies in individuals who inhale fungal spores. Therefore, it was important to use the biological safety hood for all fungal transfers. The Baker Biogard Hood, Class II, Type 1, (manufactured by the Baker Co., Biddeford, Me.) used, was designed to reduce and eliminate airborne particles which could infect personnel and contaminate desks, and glassware in the immediate area. It contains a HEPA filter designed to remove 99.97% of the particles having an average diameter of 0.34 μ m.

2. Gas-Injection Chromatography

Gas analyses were conducted on the Perkin-Elmer 900 gas chromatograph equipped with a thermal conductivity detector, using helium carrier gas at a flow of 55 cc/minute, with a detector temperature of 250 C and 175 ma current.

The column was an Alltech CRT #8700 column (Alltech Associates, Arlington Heights, Illinois) held isothermal at 35 C. Three milliliters volumes of samples were compared to the same volume of a standard gas. Gas samples from the six University of Cincinnati lysimeters and from the methane growth vials were assayed by this instrument.

3. Psychotherm Incubator Shaker

The Psychotherm Incubator Shaker (New Brunswick Scientific Co., Inc., New Brunswick, New Jersey) was used as the incubator shaker for growing both the methane utilizing and methane producing bacteria. Inside the incubator-shaker the growth vials, secured by clamps, rotated 148 times per minute in a one inch diameter circle. The incubator temperature was 35 C.

CHAPTER 4

EXPERIMENTAL METHODS

A. INTRODUCTION

The purpose of this research was to enumerate, isolate and identify the microorganisms present in the solid waste, and leachates obtained from lysimeters, landfills and soils. Of particular interest were pathogenic bacteria, fungi and fecal indicator bacteria. In addition some chemical assays were made to document the chemical environment of the landfill.

The procedures used to carry out these assays are presented under the following headings: General Microbial Procedures; Collection and Treatment of Samples; Microbial Isolation and Identification; Significance of Microbial Isolates, and Chemical Methods.

B. GENERAL MICROBIAL PROCEDURES

1. Laboratory Procedures

The laboratory procedures used in this study such as inoculating techniques, cleaning glassware, sterilizing techniques, preparation of growth media, reagents and stains, and safety techniques are consistent with those described in The Manual of Microbiological Methods by the Society of American Bacteriologists (1957), Standard Methods (1976),

Bordner et al., (1978), and Lennette et al., (1974), (1980), Koneman et al., (1979), and Washington, (1981).

2. Microbial Culture Media and Test Procedures

Selected growth media, reagents, stains, biochemical tests, and serological tests used in this study are described and referenced in Appendix B and Appendix C.

3. Buffer and Diluents

Unless specified otherwise, the phosphate buffer dilution water described in Standard Methods (1976) was used throughout this study for diluting leachate, solid waste, and other inocula for microbial enumeration and other analyses. However, for the anaerobe studies, particularly for the clostridia, peptone dilution water containing 1% Bacto-peptone (Difco) was used to dilute the inocula (Duncan and Harmon, 1976). Distilled water was used to dilute leachate, solid waste and soil samples for the pH, conductivity, and other physical or chemical assays.

4. Preparation of Standardized Inocula

The bacterial cultures were grown on brain heart infusion agar slants at 37 C for 24 hours. The cells were removed from the agar and suspended in phosphate buffer. These cells were washed three times in buffer and centrifuged at 2,000 rpm for 15 minutes to remove the buffer. The

cells were then suspended in buffer and examined for their optical density using a Klett-Summerson photoelectric colorimeter, model 800-3 using a green filter giving a wavelength of 500 to 570 μ m . (Klett Manufacturing Co., Inc., New York, New York). Readings on several dilutions were made and plate counts were made of each dilution. Then the plate counts were plotted against the optical densities. Thereafter, microbial densities were prepared which had an optical density of 0.02 or 0.04. (Cruickshank et al., 1975).

5. Reference Cultures

The purpose of reference cultures was to control the biochemical and serological reactions used in the microbial identification tests. These cultures, obtained from private collections and from the American Type Culture Collection, are listed in Table 17. They were stored in skim milk at -80 C or kept at room temperature in deep heart infusion slants except for the Clostridium perfringens and mycobacterial strains. The former were stored in cooked meat medium under oil at room temperature and the latter were kept on Lowenstein-Jensen's slants at 4 C.

6. Storage of Cultures

Most aerobic bacteria isolated from leachate, and solid waste were maintained on heart infusion agar slants. These slants contained 8 ml of agar in 13 X 100 mm glass

TABLE 17. REFERENCE CULTURES USED TO CONTROL
BIOCHEMICAL AND SEROLOGICAL REACTIONS

Species	Source or ATCC ^a Number
<u>Candida albicans</u>	ATCC #E10231
<u>Clostridium perfringens</u>	ATCC #E3624
<u>Enterobacter aerogenes</u>	ATCC #13048
<u>Escherichia coli</u>	ATCC #11229
<u>Escherichia coli</u>	ATCC #13706
<u>Klebsiella pneumoniae</u>	P.V. Scarpino ^b
<u>Klebsiella pneumoniae</u>	ATCC #E13883
<u>Mycobacterium fortuitum</u>	ATCC #6841
<u>Mycobacterium gordonae</u>	ATCC #14470
<u>Mycobacterium kansasii</u>	ATCC #12478
<u>Pseudomonas aeruginosa</u>	P.V. Scarpino
<u>Pseudomonas aeruginosa</u>	ATCC #E10145
<u>Salmonella typhi</u>	ATCC #19430
<u>Salmonella typhimurium</u>	H. Manning ^c
<u>Salmonella typhimurium</u>	ATCC # 23564
<u>Shigella boydii</u>	ATCC #E9207
<u>Streptococcus faecalis</u>	P.V. Scarpino
<u>Streptococcus faecalis</u>	ATCC #E19433
<u>Streptococcus pyogenes</u>	ATCC #E14289
<u>Streptococcus salivarius</u>	ATCC #E13419

a = American Type Culture Collection, Rockville,
Maryland 20852 - 1776.

b = University of Cincinnati

c = USEPA, Cincinnati, Ohio

screw-capped tubes which had been slanted to produce a deep butt. The cultures were streaked and stabbed on these slants, incubated at 35 C for 24 to 48 hours, and maintained at room temperature. When possible the tops were dipped into melted paraffin as an aid to retain the moisture within the tube. Most bacteria survived 2 to 3 years in this manner (Cruickshank, et al., 1975). This method of keeping isolated strains of bacteria was preferred over the method used previously when broth cultures of isolated strains were inoculated to skim milk and frozen at -80 C. The frozen milk method was finally discarded when the Salmonella typhi (ATCC #19430) lost its Vi antigen after being frozen (-80 C) in skim milk for a year or two. Anaerobes were cultured in cooked meat medium containing a 1/2 inch layer of sterile mineral oil on top of the medium to prevent oxygen diffusion. These cultures were stored at room temperature. Finally, yeast and fungi were grown on Sabouraud agar slants and also maintained at room temperature.

7. Safety Procedures

All of the solid waste and leachate, presumably containing pathogens, were handled with sterile tongs. Also, propipettes were used in transferring samples. The waste was homogenized by the Waring blender in the biological safety hood to prevent any contaminated spray from

entering the air. Transfers of the fungi and the mycobacteria were also done in the safety hood as they cause infections when inhaled. Finally, the discarded leachate was disinfected with 3% phenol and incubated at room temperature for 5 to 7 days to kill any pathogens prior to its being discarded.

8. Anaerobic Cultivation Technique

All media used for the cultivation of anaerobes were freshly prepared. When tubed media was older than 24 hours, it was placed in boiling water for 10 minutes and then cooled prior to inoculation. Before incubation plates and tubes were placed in a GasPak (BBL) unit with a BBL Disposable Hydrogen-Carbon Dioxide generator envelope; except when, as an alternate method, mineral oil was added to the tubed media (Society of American Bacteriologists, 1957).

C. COLLECTION AND TREATMENT OF SAMPLES

1. Leachate Collection

All leachate samples were collected from the lysimeters and landfills in sterile, glass, one liter bottles with screw caps, previously gassed with nitrogen to decrease the oxidation of the leachate by the air (Society for American Bacteriologists, 1957). These bottles were filled to the top with the leachate to eliminate any air pockets. On a few occasions the leachate was added to bottles containing

EDTA (A disodium ethylene-diamine tetraacetate) in order to bind the metals present in the leachate. The bottles for this purpose were prepared by adding a measured amount of the EDTA to the bottle prior to its sterilization in the autoclave. At the sampling site a definite amount of leachate was added to the prepared bottle to produce the desired molarity of EDTA and the contents were mixed (Glotzbecker, 1974). The bottles were then placed in an ice chest (5 C) for the trip back to the laboratory. There they were transferred to a 5 C refrigerator until they were processed. All leachate samples were processed within 24 hours.

2. Solid Waste Collection

The solid waste and soil samples, 1 to 3 kg in size taken from landfills and control areas were placed immediately in sterile plastic bags, and the bags were squeezed to remove the air. When anaerobic conditions were desired, the samples were kept in a GasPak (BBL) jar. All samples were placed in an ice chest for transportation back to the laboratory. There the samples were placed at 5 C and processed within 24 hours. However, for the methane studies from the Boone County landfill, TC-1, the samples were kept for three months in GasPak jars at 20 C while work was completed on the enumeration and the identification of pathogen from the leachate and solid waste.

3. Pretreatment of Samples Prior to Microbial Enumeration

a. Solid Waste Homogenization

The solid waste samples were homogenized with dilution water prior to the enumeration process. The samples had been stored aerobically at 5 C or anaerobically in a GasPak (BBL) jar at 5 C or 20 C. The samples were removed from the storage and weighed. Twenty grams of the solid waste or soil were then placed in the Waring blender jar together with 180 ml of sterile distilled water or sterile phosphate buffer. The distilled water was used in preparing the samples for pH, conductivity and other chemical determinations while the phosphate buffer was used to prepare the samples for the microbial assays. The Waring blender was run at high speed for 15 seconds in the biological safety hood. Then the blended material was allowed to sit undisturbed for 15 to 20 minutes to give the atomized liquid particles a chance to condense. Finally, the blender jar was removed from the hood, and the contents were mixed, and measured for the microbial and chemical analyses (Peterson, 1973).

b. Concentration of Microorganisms from Leachate

The use of diatomaceous earth filtration as a microbial concentration procedure was suggested by Peterson

(1973) for leachate, and for water and wastes by Bordner et al., (1978). The process, as used in this study was designed to concentrate leachate samples & was designed for the enumeration of mycobacteria and clostridia. This concentration-filtration was carried out using a membrane filter pad (45 mm in diameter) in the membrane filter apparatus. The filter was prepared by adding 10% aqueous suspension of diatomaceous earth (Celite 545, Fisher Scientific Co., Fair Lawn, New Jersey) to the apparatus to produce a filter 10 to 20 mm in height. To this filter two liters of leachate was then added. When the filter became clogged the top portion of the filter was removed with a sterile spatula and placed in 40 ml of the filter suspending medium, and the filtration was then continued. For aerobic microorganisms, phosphate buffer was used as the suspending medium; and for anaerobes, thioglycollate 135 C (Difco) or 1% peptone water was used. Such diluents used for anaerobic bacteria were placed in boiling water for ten minutes to drive out the oxygen and were cooled prior to use. When the filtration was complete the top 5 to 6 mm of the celite was placed in the suspending medium and the contents were mixed. This mixture was placed in the refrigerator (5 C) for 45 minutes to one hour to allow the celite particles to settle prior to sampling for plating the Mycobacterium and Clostridium species. It was

necessary to remove the celite particles as they could damage the agar plate surface during the plating process and could interfere with colony development afterwards during incubation. We assumed that the bacteria would remain in suspension for a longer time than the celite particles as they were much lighter. Thus, the mycobacteria and the clostridia would remain in the top portion of the mixture while the celite settled.

The concentration of the leachate produced by this process was 100 X. Thus, the 2,000 ml of the original volume was reduced to 20 ml in the suspending medium-celite mixture. This material was then plated for clostridia or was digested as described in the following section for mycobacterial enumeration and plated for mycobacteria.

c. Digestion of Sample for Mycobacterium Growth Studies

The purpose of the leachate digestion procedure was to decrease the numbers of rapidly growing saprophytes and to allow any slow growing mycobacteria if present to multiply on laboratory media. For this digestion process a one-to-one mixture of the leachate preparation (10.0 ml) and n-acetyl-cysteine-NaOH (10.0 ml) was made. They were allowed to react at room temperature for 15 minutes. Afterwards, phosphate buffer (30.0 ml) at pH 6.8, was added to neutralize the NaOH and to stop the digestion process. Then this

mixture was centrifuged at 2,000 rpm for 15 minutes to concentrate the mycobacteria in the sediment. The supernatant was then poured off, and 2.0 ml of 0.2% bovine albumin was added to the sediment to act as a mycobacterial growth stimulant. It should be noted that this last centrifugation produced an additional concentration factor of 10X. This factor of 10X together with the concentrated leachate factor of 100X from the diatomaceous earth filtration, gave a total concentration of 1000X. The sediment concentrated 1000X was then inoculated on to Lowenstein-Jensen slants and Middlebrook 7H10 agar plates using the undiluted and a 1:10 dilution of the cells. (Vestal, 1976). The incubation of these plates and their mycobacterial enumeration are described in the following section, part 2.d.

D. MICROBIAL ENUMERATION AND ENRICHMENT PROCEDURES

The leachate and solid waste assay procedures described below include: the most-probable-number tests; plate counts; and enrichment procedures (Table 18).

1. Most Probable Number Procedures

a. Total and fecal coliforms

Total coliform and fecal coliform densities were determined by the Most Probable Number (MPN) procedure as described in Standard Methods for the Examination of Water and Waste Water (1976). This was found to be the most

TABLE 18. ENUMERATION AND ENRICHMENT MEDIA

Type of Media	Bacteria & Fungi Selected for Enumeration & Isolation
<u>Enrichment Broths and Slants^a</u>	
Lauryl Sulfate broth ^b	Total Coliforms and Fecal Coliforms
Brilliant Green Bile 2% ^b	Total Coliforms and Fecal Coliforms
EC Medium ^b	Fecal Coliforms
Azide Dextrose broth ^b	Fecal Streptococci
Ethyl Violet Azide broth ^b	Fecal Streptococci
Dulcitol Selenite broth	<u>Salmonella</u>
Tetrathionate	<u>Salmonella</u>
Selenite F medium	<u>Salmonella</u>
Iron Milk medium ^b	<u>Clostridium</u>
Cooked Meat Medium	Anaerobes
Lowenstein-Jensen Egg Slant	<u>Mycobacterium</u>
<u>Agar Plates</u>	
Bile Esculin	<u>Streptococcus</u>
Blood, Aerobic	Aerobes
Blood, Anaerobic	Anaerobes
Brain Heart Infusion	Aerobes
Eosin Methylene Blue	<u>Klebsiella</u>
Inhibitory Mold	Systemic Fungi
KF Streptococcal	<u>Streptococcus</u>
Mycosel	Dermatophytes
m-Enterococcus	<u>Streptococcus</u>
Sabouraud	Fungi
Tellurite	<u>Corynebacterium</u> , <u>Listeria</u> , etc.
Middlebrook 7H10	<u>Mycobacterium</u>
Tryptone Sulfite Cycloserine	<u>Clostridium</u>

a = see Appendix B

b = used for most probable number assays

suitable method for the enumeration of coliform organisms in solid waste effluents in a comparative laboratory study (Smith and Madison, 1972), was recommended by Peterson (1973), and was found to be superior to the membrane filter method in determining bacteria numbers (including coliforms and others) in leachate by Glotzbecker (1974), and by Glotzbecker and Novello (1975). The results of the multiple tube procedure provided an estimate of the number of total and fecal coliforms by inoculation of decimal dilutions of the sample into 5 tubes of broth per dilution. This medium was lauryl sulfate broth containing fermentation tubes. Positive tubes in the presumptive test were indicated by the presence of gas in the fermentation tubes after 37 C for 48 hours. But negative lauryl sulfate tubes were not always true indicators of the presence of coliforms, as gas production was often suppressed. Allen et al., (1976) found that the transfer from all cloudy tubes gave positive EC and brilliant green bile broth results, not encountered previously using regular Standard Methods (1976) techniques of transferring only those positive lauryl sulfate tubes containing gas. Their work indicated that high microbial counts interfered with the coliform tests. Thus, in the present studies for the confirmation test; two drops of all lauryl sulfate culture, whether positive or not, were transferred at the end of the incubation period to EC broth and to brilliant

green bile broth. See Table 19 for test operating conditions.

b. Fecal streptococci

The densities were determined by the Most Probable Number (MPN) procedure as described in Standard Methods for Examination of Water and Waste Water (1976) using the five-tube technique. The multiple-tube procedure estimated the number of fecal streptococci by the inoculation of four decimal dilutions (usually 10.0, 1.0, 10^{-1} , 10^{-2} ml) of the sample into broth tube media. Positive tubes in the presumptive test were indicated by turbidity brought about by growth in azide dextrose broth after incubation at 37 C for 48 hours. Confirmation of the presence of fecal streptococci was accomplished by transferring an inoculum of two drops from each of the positive tubes into tubes of ethyl violet azide broth and incubating them at 37 C for 48 hours. The presence of fecal streptococci in the confirmed test was noted by the formation of purple growth at the bottom of the tube. The positive test for fecal streptococci in the ethyl violet azide tube was confirmed by finding Gram-positive cocci in the purple growth. The stain was made following an incubation period of 48 hours. Usually a Gram stain is made at 18-24 hours to determine the Gram staining reaction of bacteria. However, here the purpose was not to determine the

TABLE 19. FECAL INDICATOR MOST PROBABLE NUMBER (MPN)
TESTS OPERATING CONDITIONS

MPN Test and Growth Medium	Temperature and Incubation Period	Positive Reaction Indicating Growth
<u>Total Coliform Test</u>		
Lauryl Sulfate broth	37 C - 48 hr	Transferred all tubes ^a
Brilliant Green Bile 2%	37 C - 48 hr	Gas in vials
<u>Fecal Coliform Test</u>		
Lauryl Sulfate broth	37 C - 48 hr	Transferred all tubes ^a
EC Medium	37 C - 48 hr	Gas in Vials
<u>Fecal Streptococcus Test</u>		
Azide Dextrose broth	37 C - 48 hr	Cloudy - growth
Ethyl violet Azide broth	37 C - 48 hr	Purple Growth
Azide Dextrose broth	37 C - 48 hr	Cloudy - growth
Ethyl violet Azide broth	37 C - 48 hr	Purple Growth ^b
<u>Modified Fecal Streptococcus Test</u>		
(a) Azide Dextrose broth	37 C - 48 hr	cloudy = growth
Bile Esculin Agar	37 C - 24 hr	Black colonial growth ^c
(b) Azide Dextrose broth	37 C - 48 hr	Cloudy = growth
m-enterococcus agar	37 C - 48 hr	maroon colonial growth ^c
(c) Azide Dextrose broth	37 C - 48 hr	cloudy = growth
KF Streptococcus agar	37 C - 48 hr	red colonial growth ^c
<u>Clostridium perfringens Test</u>		
Iron Milk medium	37 C - 48 hr (GasPak jar)	Digestion, clotting, Gas Production

a = Allen et al. (1976)

b = Examined microscopically for Gram-positive cocci

c = Examined selected growth microscopically

Gram reaction but to detect the presence of streptococcal cells. Also, at 48 hours and beyond, these streptococci were always Gram-positive. Thus, it would seem to be appropriate to check the staining reactions at the end of the test.

For comparative purposes only and to determine the number of the streptococci in leachate, a total of five MPN tests were used. See Table 19 which lists four of the tests; the fifth is described below. All of the tests used azide dextrose broth as the first step. For the second step, tests #1 and #2 used the ethyl violet azide purple growth to indicate the presence of Gram-positive cocci. Test #1 was the Standard Methods (1976) test and test #2 was an adapted Standard Methods (1976) procedure requiring the examination of the ethyl violet azide purple growth for the presence of Gram-positive cocci (described above). In tests #3 through #5 a loopful of growth from each azide dextrose tube was placed upon designated areas of each bile esculin, m-enterococcus, and KF streptococcal agar plates. These plates were incubated at 37 C for 24 to 48 hours. Positive tests were indicated by the colored colonial growth characteristic of the three plates listed above, namely black, maroon, and red, respectively. Finally, the number of positive results were compared to the Standard Methods (1976) statistical most-probable-number (MPN) tables to determine

the concentration of streptococci in the leachate.

c. Interpretation of the fecal coliforms (FC)/
fecal streptococci (FS) ratio

This ratio has been proposed as a means of estimating whether or not the fecal pollution originated from a human or a non-human source (Geldreich, 1966; Geldreich and Kenner, 1969; Geldreich et al., 1968). Thus, a FC/FS ratio of less than 0.7 is believed to indicate fecal contamination from domesticated farm animals, whereas a ratio of greater than 4 indicates a human source. Blannon and Peterson (1974) pointed out, however, that the FC/FS ratios must be applied carefully since these correlations may be altered because of the various ecological factors existing in solid waste landfills. For example, they noted in leachates a rapid die-off of fecal coliforms and the survival of fecal streptococci. It was suggested that the use of a ratio relationship for leachates may be valid only during the initial leaching period. Feachem (1975), however, presented data that showed that the differential die-away of fecal coliforms and fecal streptococci can, in fact, strengthen the value of the FC/FS ratio as a means of distinguishing human from non-human pollution. Thus, reliance on the FC/FS ratios is possible even with differential survival. However, Feachem based his observation on FC/FS ratios in the water environment. It would also be significant if his observations could be

extended to the landfill environment where changes in FC/FS ratios also occur. In this research, these studies were directed towards differentiation of human and non-human warm-blooded animal pollution sources through comparison of fecal coliforms and fecal streptococci population density ratios. Feacham (1975) stated, that a pre-dominately human source will exhibit an initially high (>4) ratio which falls, and non-human sources show an initially low ratio (<0.7) which subsequently rises. Any other results would have an uncertain connotation.

d. Interpretation of fecal indicator levels

In examining the lysimeter and landfill leachates, an evaluation of the levels is required. However, the importance of the particular levels of the indicators in landfill leachate is not definitely known. If leachate with high fecal indicator numbers flowed upon the ground it probably would not become a public health problem. On the other hand, if the leachate levels were high and it drained into drinking water sources, then the levels would become very important. However, in the absence of a standard, the fecal indicator levels given by Geldreich (1970) will serve as a guide. Geldreich stated that 10^2 fecal coliforms most-probable-number (MPN)/100 ml in recreational water indicated the presence of Salmonella in roughly 25% of the water samples. He also stated that greater than 10^2 fecal strep-

tococci in 100 ml of recreational water would indicate fecal pollution. Also, he cautioned that some or most of the streptococci could be Streptococcus faecalis var. liqueifaciens, which are ubiquitous and would not indicate fecal pollution as they are found on plants and do not originate in humans. He gave an example of a drinking water well containing 8.0×10^3 /100 ml of fecal streptococci most of which were S. faecalis var. liquefaciens. On the other hand S. faecalis was the more resistant of the fecal pollution agents when Geldreich placed them in storm water at 20C. The agents he used included S. faecalis, S. faecalis var. liquefaciens, Salmonella typhimurium, S. bovis, fecal coliforms, and Enterobacter aerogenes. Therefore I shall consider as "high counts" those counts which are greater than or equal to 10^2 fecal coliforms and 10^3 fecal streptococci per 100 ml of leachate.

e. Clostridium perfringens

Stormy fermentation of iron milk was used as an MPN fecal indicator test in this research to determine the number of anaerobes, in particular the number of Clostridium perfringens present in landfill leachate and solid waste. The medium chosen was skim milk to which a few grains of iron filings were added prior to autoclaving the milk (Bonde 1963; and Dowell & Hawkins, 1976). This was a five tube MPN

test using four dilutions. The dilutions included the following in 1.0 amounts: 1.0, 0.1, 10^{-2} , and 10^{-3} ml. These dilutions were prepared with 1% peptone water (Duncan and Harmon, 1976). After the samples were added to the freshly heated milk tubes, all tubes were placed into GasPak jars (BBL) and incubated at 37 C for 48 hours.

A test was considered to be positive when the milk in the tube showed stormy fermentation, i.e., extensive clotting, and digestion.

As the stormy fermentation can be brought about by several clostridia, it was necessary to isolate Clostridium perfringens from several positive tubes and make a definite identification. This was accomplished by using biochemical and serological procedures which will be described in the identification section. The presence of C. perfringens was demonstrated in two out of every five positive tubes. The number of positive tubes per dilution were then compared to the MPN statistical table in the Standard Methods (1976) to determine the concentration of C. perfringens (MPN/100ml).

2. Plate Count Procedures

All samples were plated in essentially the same manner. Although many types of plating media, two kinds of buffer, and various incubation temperatures were used, all plates were inoculated by the spread plate technique. Thus,

phosphate buffer (Standard Methods, 1976) was used to dilute aerobic and on occasion anaerobic samples. However, later in the study using the tryptone sulfite cycloserine (TSC) plate inocula, 1% peptone water was used as the diluent for anaerobes. All tubes were mixed on the vortex mixer, prior to sample removal. Then, a measured 0.1 ml sample from each dilution were placed on duplicate plates and spread with a sterile bent glass rod (Bordner et al., 1978; Koch, 1981). Following this, the plate was allowed to remain upright at room temperature for one-half hour to allow the inoculum to drain into the agar. The plates were then inverted and placed in the 37 C incubator or left at room temperature depending upon the desired incubation conditions. Then all plates were incubated at 37 C for 48 hours except with the following exceptions: the bile esculin agar was incubated at 37 C for 24 hours and the Middlebrook 7H10 agar was incubated for six weeks. All anaerobic plates were incubated in the Gaspak (BBL) jar at 37 C for 48 hours with the exception that tryptone sulfite cycloserine (TSC) agar was incubated for 24 hours. The fungal plates were incubated at room temperature (23 C to 25 C). Following the incubation the plates were counted using the Quebec Colony Counter, Model 3330, made by the American Optical Company, Buffalo 15, New York (Standard Methods, 1976). The fungal plates were incubated at room temperature for one week and counted.

a. Total Plate Counts

Three media were used to determine the total plate counts during this study, although only one was used at any particular time. Standard Method agar was chosen over trypticase soy agar and brain heart infusion as it provided the highest plate counts and the inhibition of spreaders. In addition both aerobic and anaerobic blood agar plates were used for comparative purposes. The aerobic blood plates would allow certain fastidious microorganisms to grow, and the anaerobic blood plates would indicate the presence of certain anaerobes, particularly Clostridium perfringens which produces a double zone hemolysis around most colonies. All plates were incubated at 37 C for 48 hours.

b. Corynebacterium and related bacteria

Tellurite agar (Bordner et al., 1978) was used for growing diptheroid-like nonsporeforming Gram-positive rods (Table 20). The colonies produced by these bacteria on this medium were black, gray with black centers, and dull white colonies. Only the black and the black and gray colonies were counted. The incubation conditions for this medium were 48 hours at 37 C. The genera usually isolated from this medium were Corynebacterium, diptheroids, Listeria, Kurthia, and in a few instances, Streptococcus.

TABLE 20. COLONY-TYPES SELECTED FOR IDENTIFICATION

Agar Plate Medium Used	Colony Description and Agar Reaction	Microbial Type Found
Blood (aerobic)	All colony types	All Gram-negative rods and Gram-positive rods and cocci
Blood (anaerobic)	Double zone hemolysis	<u>Clostridium</u>
TSC ^a (anaerobe)	Large moist colonies Lecithinase production Lipase production	<u>Clostridium</u>
Tellurite	Black	<u>Corynebacterium</u>
Eosin methylene blue	Large, mucoid, dark or transparent	Gram-negative rods
MacConkey	transparent, red	Gram-negative rods
Inhibitory mold	Yeasts and molds	Systemic fungi
Mycosel	Yeasts and molds	Dermatophytes
Sabouraud	Yeasts and molds	All fungi
Middlebrook 7H10	<u>Mycobacterium-like</u> ^b	<u>Mycobacterium</u>
Bile esculin	Black	Fecal streptococci
m-Enterococcus	Maroon	Fecal streptococci
KF streptococcal	Red	Fecal streptococci

a = Tryptone sulfite cycloserine

b = Vestal (1976)

c. Clostridium perfringens

Several agars (Table 20) were tested during the experimental work for enumerating C. perfringens before tryptone sulfite cycloserine agar (TSC) was finally selected (Dowell and Hawkins 1976; Angelotti et al., 1962; and Duncan and Harmon, 1976). This medium was chosen because surface colonies of Clostridium perfringens could be distinguished from other clostridial colonies and could be counted. Inoculated plates of this medium were incubated at 37 C for 24 hours in a GasPak (BBL) jar. The C. perfringens colonies were large (3-5 mm), raised, glistening, and yellow surrounded by a lecithinase precipitate zone. The lecithinase zone was often 1 cm in diameter. To insure that these colonies were C. perfringens, five colonies per plate were identified by the biochemical and the serological procedures described in the following section on the identification of clostridia.

d. Gram-negative rods

MacConkey agar was used on occasion to give the total Gram-negative rod counts. However, the agar is restrictive and does not allow all pathogenic organisms to grow. It inhibits some strains of Moraxella, Acinetobacter, and Vibrio (Koneman et al., 1979). On the other hand, methylene blue (EMB) allows additional genera to grow, such as Bacillus sp. and fungi. Also this medium was selected

because Gunther (1976) indicated that Klebsiella pneumoniae could be easily recognized on this medium by their mucoid colonies. Therefore, EMB was used throughout the experiment. Finally, pseudosel (Difco) assays, used only in the beginning, were discontinued as Pseudomonas sp. is not infectious.

e. Fungi

Three types of plate media were used for fungi. First, Emmon's modified Sabouraud (Difco) agar plates were used to grow all yeasts and molds colonies (Emmons et al., 1977; Jan Schwartz, 1978, Personal Communication). The yeast colonies on this medium could be distinguished from the bacterial colonies as the former yeast colonies were white, opaque, and gave a ground glass appearance, while the latter bacteria were often shiny and translucent. The mold colonies were rough, had a velvet appearance, fluffy in texture and therefore easier to recognize (Silvia-Hunter and Cooper, 1974). The second medium, mycosel (BBL) agar, was used to grow the dermatophytes. The inhibitory mold agar (BBL) was also used to grow the deep seated molds. All fungal plates were incubated for one week at room temperature and counted.

f. Mycobacterium plate counts

As digested leachate preparations used for plating

Mycobacterium yielded only an occasional isolate, a concentration step was added to the procedure. The concentration of leachate by filtration using diatomaceous earth (as described above) was followed by a digestion process using N-acetyl-L-cysteine-NaOH to destroy the nonmycobacterial cells. Then 0.1 ml of inoculum was placed on the Middlebrook 7H10 agar plates. The inoculated plates were placed in the 37 C incubator for 24 hours right side up to allow for the inoculum to absorb into the agar. Afterwards, the plates were inverted and placed in a plastic bag to help retain the moisture during the 6 week incubation period at 37 C. All plate counts were made at 4 weeks although the plates were examined weekly for Mycobacterium colonies (Vestal, 1976). Then these Mycobacterium-like colonies were stained with the Ziehl-Neelson stain (Koneman et al., 1979) to determine their acid-fast reactions.

g. Fecal streptococci

Three differential agars were used to determine the levels of streptococci present in leachate and solid waste. These differential agars produced colored colonial growth: bile esculin (black), m-enterococcus (maroon), and KF Streptococcal (red). Inoculations of the samples were made using the spread plate procedure. They were incubated at 37 C for 48 hours and counted, with the exception of the bile esculin agar plates, which were incubated only 24 hours.

3. Enrichment Broths for Growing Salmonella, Clostridium, and Methane Bacteria

a. Salmonella

Fifty milliliters of leachate or 5 g of solid waste were added to 50 ml of double and of single strength enrichment broths, respectively. The broths used were selenite F, (BBL), GN (Difco), and tetrathionate (Difco). Later in the study a pre-enrichment broth using brain heart infusion broth (Difco) was used. For this process a 1:2 dilution of the leachate was prepared in the medium and incubated at room temperature (25 C) for two hours. Afterwards it was inoculated into the enrichment media as described directly above. The enrichment broths were incubated at 37 C for two days. Twice daily for 48 hours the broths were streaked on xylose lysine deoxycholate (XLD) agar or on Hektoen enteric agar plates and incubated at 37 C for twenty-four hours. Every coliform-type colony was streaked for isolation and inoculated into triple sugar iron agar (TSI) or Kligler's iron agar (KIA) and on lysine iron agar (LIA). The cultures that gave an alkaline slant and an acid butt with the TSI or the KIA, and a "no change" reaction with the LIA was tested with Difco's Salmonella O antiserum, Poly A and Poly B, and Salmonella Vi Antiserum. Cultures which have positive agglutination test were considered to be Salmonella isolates. API 20E was also used.

b. Clostridium

Five milliliters of leachate or one milliliter of a concentrated leachate preparation were inoculated into tubes of cooked meat or iron milk media. All tube media had previously been heated to boiling temperatures for 10 minutes and then cooled. After the tubes had been inoculated they were either overlayed with sterile mineral oil or were placed in a GasPak jar. The tubes were then incubated for at least two weeks at 37 C until some evidence of anaerobic growth was observed such as blackening of the meat, cloudy growth, or a digestion of the milk with gas production. Following the appearance of anaerobic growth, the tubes were Gram stained and streaked on a blood agar plate, which was incubated in a GasPak (BBL) jar for 24 hours at 37 C. The culture was then examined for double hemolysis. If no double hemolysis was present it was then placed in the 5 C refrigerator for two to four hours to enhance the hemolysis. If no double hemolysis was present the Clostridium isolates were identified by the biochemical methods described later in this section.

c. Methane utilizing and methane producing bacteria

Initially, two enrichment media were used to grow the methane-utilizing bacteria. Later, one was selected for future use. These were Brown's medium (Brown et al., 1964), and Leadbetter's medium (Leadbetter and Foster, 1958). The

components of these broths are shown in Appendix B.

The methanogenesis medium (See Appendix B) was adapted from those media described by Paynter and Hungate (1968), Zeikus and Winfrey (1976), and by Strayer and Tiedje (1978). Sewage sludge was used in place of the rumen fluid (Pfeffer, 1974). The medium was sterilized and placed in glass vials. Nitrogen gas was added to the medium before and after the sterilization. Before inoculations were made of the sample, 0.25 ml of a 50% NaHCO_3 solution was added to each vial together with 0.5 ml of an 18-24 hour culture (brain heart infusion broth) of Escherichia coli, ATCC 13706. These vials were then flushed with purified nitrogen gas and incubated overnight at 30C to allow the coliforms to utilize the oxygen (Strayer and Tiedje, 1978).

After the vials were inoculated with the solid waste, N_2 , CO_2 , or CH_4 purified gases natural gas or air, depending upon the experiment, were used to fill the individual vials prior to insertion of the serum sleeve. Afterwards a second gas, if needed, was added to the vial using a luer lok syringe. It was necessary to obtain the correct gaseous atmosphere (Alexander, 1961; Whittenburg, et al., 1970; Wolf, 1970; and Foster and Davis, 1966). For example, methane-using bacteria required CH_4 and air, and methane-producing bacteria required anaerobic conditions, CO_2 , and H_2 . For these purposes purified gases were used in the

experiment except during the preliminary experiments when the methane gas was unavailable. At that time the natural gas from the laboratory burner jets was used. At all other times the Scott Specility Gases (Bellefonte, Pennsylvania) were used in the culture vials: hydrogen, No. 3-00-100, 99.95% pure; Carbon dioxide, No. 2-3402, 99.8% pure; and methane, No. 2-2562, 99.0% pure. See Appendix D.

After the gas additions, the culture vials were incubated at 35 C for several weeks in an incubator-shaking machine. At periodic intervals the vials were removed from the incubator for the purpose of releasing the excess air pressure, to measure the gas-uptake, and to "feed" the required gases to the cultures. Before gases were added to the vials or the gases removed, they were allowed to come to room temperature. Then the gas in the vials containing high gas pressure would be released, and the amount recorded. Following this, the gas-uptake and gas additions were made. The gas-uptake for a particular vial was always a portion of the gas volume added. Gas-uptake was measured at several times during the incubation period to determine the utilization of gases in the vial. The purified gases listed above were usually "fed" to the vial cultures at least once every two weeks, at which time the gas-uptake was measured. This process involved the filling of a sterile 10 ml syringe with CH_4 , CO_2 or H_2 and inserting the needle into the vial.

When the gas volume in the vial was decreased, gas from the syringe would be passively transferred to the vial. This uptake was measured and recorded. To accomplish this the vial and the attached syringe were laid flat on the table to eliminate the pull of gravity on the syringe plunger. Then, the uptake was recorded and the rest of the gas in the syringe was added to the vial. For example, if 7 ml of gas was taken into the vial by passive action this was called gas-uptake, and if the protocol called for 10 ml to be inserted that day, then the other 3 ml of gas would be added to the vial after the amount of uptake was noted.

Gas analyses were conducted on a Perkin-Elmer 900 gas chromatograph (See Section 4). For the analysis 3 ml volumes of sample gas were compared to the same volume of a standard gas. The standard used was Standard Scott Gas #9800 (Scott Environmental Technology, Inc., Bellefonte, Pennsylvania) containing CH_4 , CO_2 , CO , O_2 , and N_2 . The analysis was normalized for 100% based on the occurrence of CH_4 , CO_2 , O_2 , and N_2 in the sample (McNair and Bonelli, 1969). A computer program was used for individual sample calculations.

E. MICROBIAL IDENTIFICATION

1. Overview

For the identification of pathogens and fecal indicator bacteria, selected isolates were picked from the

enumeration agar plates and of different agar plates following streaking of enrichment cultures (Table 20). These isolates were streaked for pure culture isolation and then identified. The pure culture isolation procedure is described directly below and the identification procedure is given under the individual microbial headings and also in Table 21.

To obtain pure cultures Gram-negative rods were subcultured and streaked for at least three times on blood agar, brain heart infusion agar, or heart infusion agar. The fungi were streaked once on Sabouraud agar. The Mycobacterium were streaked twice on Middlebrook 7H10 agar. Clostridium sp. isolates were streaked twice on egg yolk agar to remove the spreaders and once on blood agar. Finally, some streptococcal cultures contained diptherids which were difficult to separate&resisted their separation from the streptococci. However, by using the following streak plate sequence pure streptococcal cultures were obtained: KF streptococcal, TTC (2,3,5-triphenyl tetrazolium chloride) and tellurite agars (Lennette et al., 1974; and Pavlova et al., 1972). Following the pure culture isolation the microbial strains were identified to genera and sometimes to species (Bergey's Manual, 1976; Cowan, 1974; Edwards and Ewing, 1962; Lennette et al., 1974, 1980; Weaver et al., 1972; Koneman et al., 1978, 1979, and Washington 1974, 1981.

TABLE 21. AGENTS USED FOR MICROBIAL IDENTIFICATION (PART I)

Media and Tests	Reference
<u>Clostridium perfringens</u> and other clostridia	
API-20A	Analytab Products, Inc Plainview, New York Washington (1980)
Blood Agar, double hemolysis	
Egg yolk Agar, test for lecithinase and Lipase	
Esculin Hydrolysis	
Gelatin Hydrolysis	
Iron Milk, stormy fermentation	
Lactose Fermentation	
Motility	
Nagler Test	
Urea Utilization	
<u>Corynebacterium</u> and Related Genera	
Blood Agar, hemolysis	Cowan (1974)
Catalase Test	
Esculin Hydrolysis	
Gelatin Hydrolysis	
Glucose Fermentation	
Lactose Fermentation	
Maltose Fermentation	
Motility (25 & 37 C)	
Nitrate Test	
O/F Oxidation/Fermentation	
Urease Utilization	
Voges-Proskauer Test	
<u>Fungi</u>	
Blood Agar, Dimorphisin Test	Weaver et al. 1972
Germ Tube Test, <u>Cryptococcus</u> Test	Koneman et al. 1979
Karung's Medium, Dimorphisin Test	
Thistle Medium	
Microscopic Examination	

TABLE 21. AGENTS USED FOR MICROBIAL IDENTIFICATION (PART II)

Media and Tests	Reference
Gram-negative Rods:	
Triple Sugar Iron Agar (TSI)	Wolf et al., 1975
Lysine Iron Agar (LIA)	
Motility Test	
Nitrate Test	
Christensen's Urea	
Cytochrome Oxidase Test	
Indol Test	
Methyl Red Test	
Voges-Proskauer Test	
Simmon's Citrate	
O/F Oxidation/Fermentation	
MacConkey Agar	
<u>Salmonella-Shigella Agar</u>	
<u>API-20E</u>	Analytab Products, Inc. Plainview, New York
O/F Oxidation/Fermentation	
Arginine Dihydrolase Test	
Cytochrome Oxidase Test	
Desoxyribonuclease Agar	
IMVIC Test	
Lysine Iron Agar	
Lysine Decarboxylase	
Nitrate Test	
Ornithine Decarboxylase Test	
Phenyl Alanine Dehydrolase (PAD) Test	
Triple Sugar Iron agar	
Urea agar	
Arginine Dihydrolase Test	Weaver et al. 1972
Catalase Test	Koneman et al. 1972
Cytochrome Oxidase Test	
Indol Test	
Lysine Decarboxylase Test	
MacConkey Agar	
Motility (25, 37C)	
Nitrate Test	
O/F, Oxidation/Fermentation Test	
<u>Salmonella-Shigella</u>	

TABLE 21. AGENTS USED FOR MICROBIAL IDENTIFICATION (PART III)

Media, and Tests	Reference
<u>Mycobacterium</u>	
Antibiotic Susceptibility	Vestal, 1976
Lowenstein-Jensen Slants	
Middlebrook 7H9 Agar ^a Broth	
Middlebrook 7H10 Agar	
<u>Streptococcus</u>	
<u>Agar Plates</u>	
Bile Esculin	Pavlova et al., 1972
heart Infusion	Bordner et al., 1978
KF Streptococcal	
Tellurite	
Tetrazolium	
<u>Biochemical and Growth Tests</u>	
Bacitracin Sensitivity	
Fermentation of Glycerol	
Fermentation of D-Sorbitol	
Growth at 10 and 45C	
Growth in 40% Bile	
Growth in 6.5% NaCl	
Growth in pH 9.6	
Growth in 0.1% Methylene Blue	
Gelatin hydrolysis	
Hippurate hydrolysis	
Fermentation of Arabinose	

2. Clostridium

Clostridium isolates came from cooked meat or iron milk enrichment broths, iron milk most probable number tubes, or from tryptone sulfite cycloserine agar plates. Following their pure culture isolation they were identified according to one of three processes:

1. They were identified as Clostridium perfringens (a lecithinase-producer) and confirmed by the Washington (1981) procedure and by the Nagler test, or
2. They were identified as one of the lecithinase or lipase positive clostridia other than C. perfringens according to the Washington (1981) procedure and the API-20A strip (Analytab Products, Inc., Carle Place, New York), or
3. They were picked as a nonlecithinase or lipase producer on egg yolk medium and identified by the API-20A. If it was a motile spreading colony on blood agar then it was picked as a possible C. tetani.

The identification of C. perfringens required first that the culture produce a stormy fermentation in milk and lecithinase on egg yolk agar. As neither of these two characteristics are specific for C. perfringens, the follow-

ing biochemical tests were also performed : lactose fermentation, gelatin hydrolysis, and motility (Dowell and Hawkins, 1976; and Washington, 1981). Finally the clostridial serological type or the identification was made by the Nagler test or by the API-20A. The Nagler test determined whether or not the C. perfringens was Type A, from humans or Type B from animals (Davis et al., 1980 and Smith and Dowell, 1980). The API-20A determined the clostridial species other than C. perfringens.

3. Corynebacterium, Kurthie, and Listeria

These bacteria were usually isolated from blood plates or tellurite agar plates. On the latter medium the colonies were small (2 mm), black and raised. These bacteria were separated from other similar genera by the scheme from Cowan (1974) and Washington (1981). Such bacteria grew faster than the mycobacteria and were further differentiated from them by their colonial morphology and by their not being acid-fast. They were differentiated from the Bacillus sp. by not producing spores. This was demonstrated by inoculating the SIM medium and incubating the culture for two days at 37 C and five days at room temperature. The spores were demonstrated by Gram stain.

4. Fungi

The Communicable Disease Center (1974) booklet, Etiologic Agents Classified on the Basis of Hazard listed Actinomyces sp., Nocardia sp., Blastomyces dermititidis, Cryptococcus neoformans, and Paracoccidioides braziliences as Class 2 agents, Histoplasma capsulatum as a Class 3 agent. However, it is our observation that the latest clinical textbooks on the identification of pathogenic yeast and molds list increasing numbers of saprophytic fungi that seem to be present in pathogenic and infectious fungal lesions. (Lennette et al., 1980; and Washington, 1981). It was for this reason that all molds were identified.

The filamentous fungi from the landfills and the lysimeters leachate and solid waste were first enumerated by counting the fungal colonies on Sabouraud agar, (Difco), mycosel agar (BBL), and inhibitory mold agar plates (BBL). They were allowed to stay at room temperature (25 C) for one week, at which time they were counted. Their incubation at room temperature was continued for four to six weeks, and at this time all the fungal types were selected, isolated and identified.

The fungi were usually separated and identified according to the scheme given in Figure 12, and using the media listed in Table 22. The yeast growth was recognized

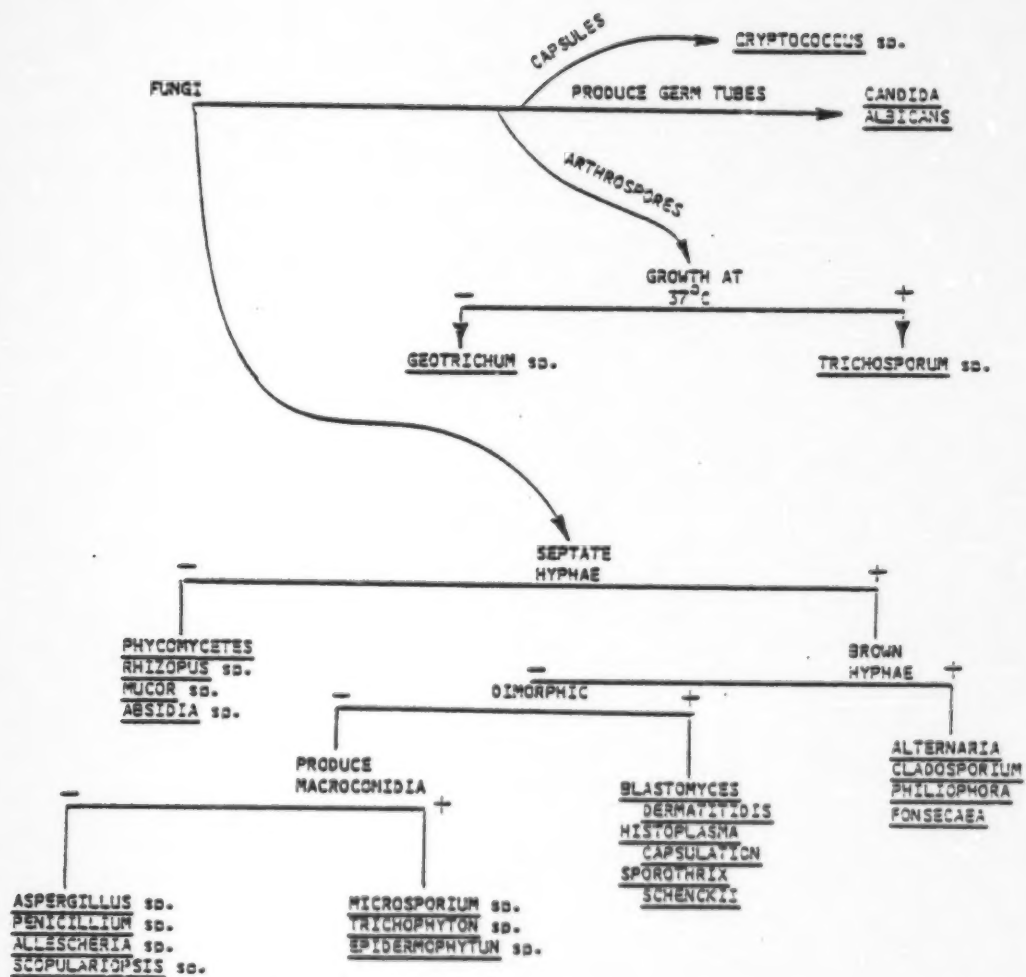


FIGURE 12. THE CLASSIFICATION OF FUNGI INTO MAJOR GROUPS
(FROM KONEMAN, 1978)



TABLE 22. MEDIA AND REAGENTS USED IN THE ISOLATION AND IDENTIFICATION OF MOLDS AND YEASTS

Media or Reagent	Purpose
Blood Agar Slants	to demonstrate dimorphism
Karung Agar Slants	to demonstrate dimorphism
Cornmeal Agar	to produce typical yeast colonies
Mycosel agar	for dermatophyte growth
Sabouraud agar (Emmon's Modification)	for yeast and mold counts
Inhibitory Mold Agar	for systemic mold growth
Sabouraud Agar Slants	for mold stock cultures
Urea Agar Slants	for <u>Cryptococcus</u>
Heart Infusion Agar Slants	for yeast stock cultures
Horse Serum	to produce germ tubes in yeast
Lactophenol blue stain	to demonstrate mold hyphae
Nigrosin	to detect <u>Cryptococcus</u> capsules
Acid-fast stain (modified for <u>Nocardia</u>)	to identify Nocardia
API-20c	to identify yeast
Birdseed agar	to identify <u>Cryptococcus</u>

by the typical colonial characteristics observed by microscopic examination. Typical yeast were given the germ tube test. Any yeast which produced a germ tube when inoculated in horse serum (or fecal calf serum) within four hr (Lennette et al., 1974) was regarded as a presumptive identification of Candida albicans. The arthrospores producing fungi were separated by further incubating them at 37 C to demonstrate the presence of Trichosporon which grows at that temperature. The molds were examined microscopically using a slide preparation made with scotch tape (Koneman et al., 1978). The clear tape, 1 to 2 cm wide, was carefully lowered to an isolated colony, near the edge of the growth, and quickly placed on a glass slide containing lactophenol aniline blue mounting dye. The slide was examined microscopically with a dissecting microscope and an oil immersion microscope. Under the microscope the hyphae were examined for color, crosswalls, type of hyphae, the arrangement of the conidia on the hyphae, etc. Several standard texts were used for the identifications, namely Lennette et al., (1974, 1980); Koneman et al., (1978, 1979); Washington (1981); Emmons et al., (1977); and Rippon (1974). When the dimorphic molds were suspected, then Karung's medium (Karung and Yegim, 1954) was inoculated from the isolating plate. This was incubated at 37 C for two to three weeks to demonstrate the possible yeast phase of the molds. The mycosel plates

were restrictive, and these were examined with the expectation that these molds would be dermatophytes. Such microorganisms were examined for the presence of macro- and microconidia. The zygomycetes were separatee from the others by noting the absence of crosswalls in their hyphae. The final classification was made by the separation of the crosswalled hyphae into those with brown or yellow hyphae from those hyaline hyphae which were without this color. The former were the dematiaceous molds as opposed to the hyaline molds. The branching of the hyphae and the arrangement of the spores usually give a definite identification of the genus following the microscopic examination.

5. Gram-Negative Rods

Several identification schemes were used. They are listed generally in the order used.

a. Wolf's Procedure

This is a classical textbook identification scheme devised for use in the hospital laboratory (Wolf et al., 1974). Initially, the cultures were divided into groups by the oxidase test and by the O/F Oxidation/Fermentation tube tests (Hugh and Liefson, 1953). The oxidase-positive and nonfermentative groups were inoculated to triple sugar iron agar, motility, indol, urea, nitrate, MacConkey, and SS media and the results were compared with their identifi-

cation scheme. The tests for the Enterobacteriaceae including the Salmonella and the Klebsiella were grouped into the lactose-positive and negative fermenters according to the Edwards and Ewing (1962) scheme.

b. API 20E

The API-20E (Analytab Products Inc., Plainview, New York) is a bacterial identification system for Gram-negative rods including the Enterobacteriaceae and the related oxidase positive (some negative) fermenters and nonfermenting bacteria. These 20 cupules placed in plastic strips were filled with a bacterial inoculum, made from a 24 hour heart infusion agar slant, and prepared by adding the bacterial cells to physiological saline to produce a density equivalent to the MacFarland nephelometer tube number one. The strips were incubated at 37C for 24 to 48 hours as indicated by the manufacturer's instructions. The reagents used to make the test determinations were obtained from the manufacturer. Finally, the API 20E profile index was used to make the bacterial identifications (Analytab Products, Inc., 1977, 1978).

c. King-Weaver, and Edwards-Ewing Methods

The fermenting Enterobacteriaceae were separated from the nonfermenting Gram-negative rods by the O/F dextrose oxidation/fermentation medium, growth on MacConkey agar,

nitrate test, and motility. In addition, the following reactions of all Gram-negative rods were determined: arginine, lysine and ornithine decarboxylases, triple sugar iron agar, lysine iron agar, motility, indol, methyl red, Voges-Prokauer, urease, deoxyribonuclease, and phenyl alanine deaminase. From these tests the non-fermenters were identified by the King-Weaver scheme (Weaver et al. 1972, and Koneman et al., 1979), and the fermenters by the scheme of Edwards and Ewing (1962), Washington (1981), and Lennette et al. (1974, 1980).

6. Mycobacterium

a. Culture Sources

Colonies on Middlebrook 7H10 agar, following leachate concentration and digestion, suggested that Mycobacterium sp. or molds were present. When growth appeared, usually within a few days to a week or so, it was restreaked on to the Middlebrook 7H10 agar plate for isolation purposes. It was then stained with the Ziehl-Neelson acid-fast stain and examined microscopically (Koneman et al., 1979). Control cultures from the American Type Culture Collection were used for comparisons: Mycobacterium kansasii (ATCC #12478); M. gordonae (ATCC #14470); and M. fortuitum (ATCC #6841). In addition soil control cultures were used. These were Mycobacterium sp. isolated from soil on the University of Cincinnati

campus and various wooded soil areas in this region of the country.

b. Identification procedures

The general identification procedures and identification schemes followed in this work were those described by Vestal (1976) and by Runyon et al., (1980).

c. Antibiotic susceptibility tests

All Mycobacterium cultures were grown in Middlebrook 7H9 broth for seven days at 37 C. Three sterile glass beads were added to each inoculum tube. The tubes were shaken on a Vortex mixer for one minute. The culture was then stained with the Ziehl-Neelson stain to determine if the cells present were acid-fast. The number of microorganisms found per field were compared to a chart from Runyon (1980) (see Table 23) listing the relation between the microorganisms per field and their concentration in Middlebrook 7H9 broth. The inoculum dilutions for the test were then prepared according to this table. It was necessary to prepare both high and low inoculum plates to insure that no more than 50 colonies per plate quadrant were present, therefore two sets of dilutions were prepared. Accordingly, the inoculum tubes were diluted as indicated in the first and second columns (Table 23). Thus, the susceptibility tests were run twice using both inoculum concentrations in order to be certain that the correct bacterial concentrations were added to the

TABLE 23. Dilutions Required for the Preparation of Susceptibility Test Inocula.

Number of Acid-Fast Bacilli/field ^b (Oil Immersion)	Use the Dilutions ^c Listed Below to Prepare the:	
	Higher Dilution	Lower Dilution
1	undiluted	10^{-2}
1 - 10	10^{-1}	10^{-3}
10	10^{-2}	10^{-4}

a = From Runyan et al. (1980).

b = From a 7 day Middlebrook 7H9 broth culture incubated at 37 C.

c = Prepared with sterile physiological saline.

antibiotic plates. See Table 24 for the mycobacterial and antibiotic concentrations used in the susceptibility tests.

To inoculate the antibiotic susceptibility plaes, 0.1 ml of the desired inoculum (high or low) dilution was placed upon all of the quadrants of all of the plates by the spread plate technique. Following this the plates were placed in plastic bags in an upright position in a 37 C incubator and read after three weeks. A positive test indicated that many colonies were present and that the mycobacteria was resistant and survived the effect of the antibiotic. A negative test indicated that the Mycobacterium was sensitive to the antibiotic as they did not grow out.

7. Streptococcus

a. Streptococcal isolation and Storage Procedures

Streptococcal growth from leachate or solid waste was obtained from azide dextrose broth, KF agar, bile esculin, m-enterococcus, or KF streptococcal agar plates. Following the pure culture isolation described initially all streptococci were maintained on heart infusion agar deeps in screw capped tubes (13 X 100 mm). For all biochemical tests the streptococci were grown for 48 hours at 37 C in heart infusion broth.

TABLE 24. ANTIBIOTIC CONCENTRATION IN AGAR^a USED FOR MYCOBACTERIAL
DRUG SUSCEPTIBILITY TESTS (FROM RUNYON ET AL., 1980)

Drug Name	Drug Concentration (Mcg/ml)			Control
	low concentration In Disc	In Agar	high concentration In Disc In Agar	
Ethambutol	25.0	5.0	50.0	0
Isoniazid	1.0	0.2	5.0	0
PAS ^c	10.0	2.0	50.0	0
Rifampin	5.0	1.0	25.0	0
Streptomycin	10.0	2.0	50.0	0

a = Middlebrook 7H10 agar in quadrant plates.

b = Drugs obtained from BBL in paper discs were placed in the bottom of each quadrant (petri dish) before the addition of the agar. Plates were then placed in the 37 C incubator for 24 hours prior to inoculation to allow the drug to diffuse throughout the agar.

c = p-aminosalicylic acid.

b. Biochemical Procedures

The biochemical procedures used for identifying the streptococci isolated from leachate and landfills included: bacitracin sensitivity, growth in bile esculin, 40% bile, pH 9.6, and 6.5% sodium chloride broths, fermentation of glycerol sorbitol, arabinose, and lactose; gelatin hydrolysis; hemolysis; hippurate hydrolysis; growth on KF streptococcal, tellurite, and TTC (2,3,5-triphenyl tetrazolium chloride) agars; starch hydrolysis; methylene blue reduction; and finally growth at 10C and 45C in broth. The sources for these tests are Pavlova et al. (1972), Bordner et al. (1978), Facklam et al. (1974), and Facklam (1980).

c. Streptococcal grouping procedures

Two streptococcal grouping procedures were used: Facklam's (1974) biochemical procedure and a serological test, Streptex, manufactured by Wellcome Reagents Ltd., Beckenham, England BR3 3 BS.

1.) Facklam's Biochemical Procedure

Facklam et al., (1974) used a few critical characteristics and were able to separate those streptococci found associated with man into the major groups without the long and involved serological procedures. The major classifications are shown on Table 25.

TABLE 25. PRESUMPTIVE DIAGNOSIS OF STREPTOCOCCI^a

Group	Hemolysis	Bacitracin Sensitivity	Hippurate Hydrolysis	Bile-esculin Hydrolysis	Growth in 6.5% NaCl	Represents % of Group
A	Beta	+	-	-	-	99.5%
B	Beta	-	+	(100%)	-	99.7%
Not Group A, B, or D (i.e., Group C, G, and/or F)	Beta	-	-	-	-	92.6%
D					(Occasionally positive)	
(Not Enterococci)	None	-	-	+	-	97.8%
Enterococci (Group D)	Alpha, Beta, or None	-	(88 = +)	+	+	99.7%
Viridans	Alpha	-	-	-	-	94.7%
		(8-120 = +)				

^a = From Facklam et al., (1974)^b = Sheep blood cells

2.) The Streptex Procedure

This procedure is a latex agglutination procedure which used six grouping sera, anti Group A, B, C, D, F, and G streptococci sera.

The antigen preparation for the test involved the suspension of the pronase solution according to the manufacturer's directions, and the addition of 0.4 ml of the solution into a 12 X 75 mm tube. To this solution was added several loopfuls of streptococcal growth. This enzyme-streptococci solution was incubated at 56C for one hour. After which the mixture was centrifuged at 2,000 rpm for 15 minutes. The supernatant was removed for the grouping test.

For the test the antigen extract, from each streptococcal strain were mixed with each of the six antisera: Group A, Group B, Group C, Group D, Group F, and Group G. The solutions were mixed until the latex agglutination was complete. A positive reaction occurred when the latex material clumped to produce a white precipitate. Facklam (1980) stated that the S. bovis reaction, prepared by an autoclaved antigen extract procedure may fail to react with the anti Group D antisera, and suggested that additional biochemical tests should be done, such as the bile-esculin test and the growth on 6.5% NaCl in brain heart infusion broth.

d. Streptococcal species determinations

The procedures used to determine the species of the fecal streptococci have been described by Pavlova et al. (1972), and recently by Bordner et al., (1978). The procedure by Pavlova et al. was recommended by and presented in Standard Methods (1976 and 1981). More information can be found in Pavlova et al., (1972) in Topley and Wilson (1974) and Bergy's Manual (1976).

e. Streptococcal Origins

Some species of streptococci are animal species specific, i.e. some are found only in man, the horse, the cow, or in fowls, while others are found in general areas such as in plants, animals, or insects. All origins of the streptococcal species in these areas are listed in Table 26. When any of these species were identified from a particular waste, the specific origin or more often the general origin of the waste were determined by finding the species on this list and noting the species origin.

F. SIGNIFICANCE OF MICROBIAL ISOLATES

As one of the objectives of this research was to enumerate, isolate, and identify pathogenic microorganisms from leachate and solid waste, a definition of pathogenicity or the criteria for determining microbial pathogenicity was

TABLE 26. THE HUMAN, ANIMAL AND VEGETABLE ORIGINS OF STREPTOCOCCAL GROUPS^a

Group ^a	Species ^b	Origin ^b
A	<u>S. pyogenes</u>	man
B	<u>S. agalactiae</u>	cattle, man
Not Groups A, B or D (i.e., Groups C, G, F)	<u>S. dysgalactiae</u> <u>S. equi</u> <u>S. equisimilis</u> <u>S. zooepidemicus</u> <u>S. anginosus</u>	cattle horse man, horse animal man
D, not enterococcus	<u>S. bovis</u> <u>S. equinus</u>	animal horse
Enterococcus (Group D)	<u>S. faecalis</u> <u>S. faecalis</u> var. <u>liquefaciens</u> <u>S. faecalis</u> var. <u>zymogenes</u> <u>S. faecium</u> <u>S. durans</u>	man, animal plants insects man, animal man, animal man, animal
Viridans	<u>S. salivarius</u> <u>S. mitis</u> <u>S. avium</u>	man man fowl
Q	<u>S. lactis</u> <u>S. cremoris</u>	Milk Products Milk Products

^a = Lancefield Groups are A through Q

^b = From Wilson & Miles (1975), except for S. faecalis var. liquefaciens which is from Goldreich, Kenner, and Kabler (1964)

^c = Bergy's Manual (1974)

important. Accordingly, three methods to determine the pathogenicity or the infectiousness of microorganisms is presented:

1. Pathogenicity can be determined in the laboratory using animals. Koch's Postulates describes the classifical procedure (Davis et al., 1980). This method is time consuming and was not be done here.
2. Pathogenicity of microbial species but not of individual strains can be determined by referring to medical textbooks or clinical laboratory manuals, such as Davis et al., (1980); Lennette et al., (1974), (1980); Koneman et al., (1979); Washington, (1981), and Bergy's Manual (1974).
3. Pathogenicity may be listed in the Communicable Disease Center (1972) booklet, "Etiologic Agents Classified on the Basis of Hazard". These agents were classified on the basis of their infectivity to laboratory personnel .
The agents included the bacteria, fungi, viruses, and parasites. See Table 27. By this classification, classes 2 and above would be considered pathogenic and infectious.

Finally, although not listed with the above methods, fecal indicator tests indicate the presence of pollution. High

TABLE 27. THE BASIS FOR CLASSIFICATION^a OF HAZARDOUS MICROBIAL AGENTS

Class	Definition
1	Agents of no or minimal hazard to human or animal health
2 ^b	Agents of ordinary potential hazard such as staphylococci, that can cause disease when the agent penetrates the skin.
3 ^b	Agents of special hazard such as human tuberculosis which require special conditions in order to contain them.
4 ^b	Agents of extreme hazard to personnel or may cause serious epidemic disease. Example: smallpox virus.
5 ^b	Foreign animal pathogens which cannot be permitted in the United States. Example: foot and mouth disease virus.

a = From Classification of Etiologic Agents on the Basis of Hazard, 1976. See Appendix D.

b = Classified as pathogenic in this report.

levels of total coliforms, fecal coliforms, and fecal streptococci indicate the presence of fecal pathogens (Standard Methods, 1976). All methods except the first have been used in this work.

G. CHEMICAL PROCEDURES

The following is a brief description of the chemical procedures performed on the leachates and solid wastes from landfills or lysimeters or wastes designated for incorporation into those units. All samples were kept in the refrigerator (5 C) until assayed. Chemical analyses were run within a week after obtaining them. Leachate samples were diluted if needed. The solid waste samples were homogenized first and then analyzed. A description of this homogenization was described earlier in this section. The procedures run on the samples, are listed in Table 28. Two instruments used for the analyses at the University of Cincinnati were the pH meter (manufactured by the E. H. Sargent Company, Model LS, Skokie, Illinois 60077), and a conductivity Bridge, Model 31. It was made by Yellow Springs Instrument Company, Inc., Yellow Springs, Ohio.

TABLE 28. TYPES OF CHEMICAL ASSAYS USED IN THIS STUDY

Chemical Assay	Procedure
Conductivity	<u>Standard Methods</u> (1976)
Chemical Oxygen Demand	EPA Methods ^a (1974)
Total Kjeldahl Nitrogen	EPA Methods ^a (1974)
Percent Moisture	<u>Standard Methods</u> (1976)
pH	Chian and DeWalle (1975)
Total Phosphate	Chian and DeWalle (1975)
Solids - Total	EPA Methods ^a (1974)
Solids - Volatile	EPA Methods ^a (1974)

a = Methods for Chemical Analysis of Water and Wastes (1974)

H. TEST PROCEDURES: PURPOSE

A description of procedures used repeatedly in this research are described in the first part of this chapter (Chapter 4) and include the following items: sample treatment, namely digestion, homogenization, and concentration; purification and storage of bacterial cultures; and microbial enumeration and identification. Procedures used on a limited basis not described in the presentation of the results including the data tables of Chapters 5, 6, or 8, will be presented here.

I. TEST PROCEDURES: DEVELOPMENT OF METHODS

1. Storage of Leachate

First, the leachate samples were diluted with phosphate buffer and 0.1 ml were plated on brain heart infusion agar using the spread plate technique (Bordner et al., 1978). The plates were incubated at 37 C for 48 hours. Each different colony type was Gram stained. The colony types were counted and recorded. Then, the leachate samples were placed in 150 ml glass bottles and frozen at -80 C. After one week, these leachate samples were placed in 25 C water for thawing. Then the samples were again plated and analyzed as described above.

2. Toxicity and Nonspecificity in MPN Tests
(Bacterial Stress Problem)

a. Streptococci from MPN Tests

Fecal streptococci enumeration and identification studies were made on the Center Hill lysimeter leachate samples (CH-1 through 19). The enumeration procedure used was the Standard Methods (1976) most probable number (MPN) procedure.

The cultivation of these same leachate samples for fecal streptococci was made by placing 1.0 ml of leachate into tubes of cooked meat medium. The tubes were incubated at 37 C for 10 days. The tube contents were Gram-stained on the second and the tenth day.

b. Gram Stain Used to Improve Specificity

Initially the Standard Methods (1976) MPN test was run. After the ethyl violet tube had incubated at 37 C for 48 hours, the results were read. All positive tubes, those that contained purple growth, were Gram stained and examined microscopically for the presence of Gram-positive cocci. No tube was considered positive unless it contained both the purple growth and the cocci. Here after all fecal streptococcal MPN tests (Standard Methods, 1976) included the Gram stain.

c. The Removal of Leachate Toxicity by
Leachate Dilution

Diluted leachate samples (phosphate buffer) in 1.0 ml amounts were placed in brain heart infusion broth (9.0 ml) and incubated at 37 C 1, 3, 6 and 24 hours. The reaction mixtures were again diluted and plated on brain heart infusion agar. The plates were incubated aerobically at 37 C for 48 hours and counted.

d. The Demonstration of Leachate Toxicity
with Plate Counts

Standardized concentrations of the following bacteria were prepared for inoculation into leachate to determine their survival time (leachate toxicity) Klebsiella pneumoniae (ATCC #13383); Pseudomonas aërsinosa (Scarpino); Salmonella typhimurium (Manning), Streptococcus faecalis (Scarpino). Cultures from overnight growth on brain heart infusion agar slants were washed three times and diluted with saline to the desired optical density. These standard inocula in 1.0 ml amounts were added to 9.0 ml of the leachate and to 9.0 ml of saline buffer for a control. The mixtures were incubated at 25 C for one-half hour. Following this the mixtures were diluted with saline and plated on eosin methylene blue, xylose lysine desocholate agar, and KF streptococcal agar plates, to enumerate Klebsiella, Pseudomonas, Salmonella, and the Streptococcus, respectively. The

spread plate technique was used for all plates in this research . All plates were incubated at 37 C for 24 hours except for the KF plates which were incubated for 48 hours. The leachate used was taken from CH-19 lysimeter on 11/17/77. The second toxicity assay was done in a similar manner.

e. The Addition of EDTA

Sterile EDTA was added to leachate at a concentration of 0.01 M in a sterile glass bottle. The bottle was shaken to mix and kept in the refrigerator until assayed. The mixture was plated on blood agar plates and incubated both aerobically and anaerobically (GasPak Jar, BBL) at 37 C for 48 hours. The total coliform and fecal coliform tests used were the Standard Methods (1976) MPN test as modified by Allen et al., (1976). The fecal streptococci test was the Standard Methods (1976) MPN test plus the Gram-stain for cocci. These fecal indicator tests were used routinely in the research.

3. The Digestion and Concentration of Leachate for Culturing Mycobacterium

Two liters of leachate per sample were concentrated upon a diatomaceous earth filter placed in a membrane filter apparatus (Bordner et al., 1978). This concentration procedure is described at the beginning of this chapter. After the filtration was complete the filter was removed from the

apparatus and the top 5 mm of the filter material was removed and placed in beakers containing 40 ml of phosphate buffer. A volume of 20 ml of concentrated leachate was produced following a settling period in the refrigerator at 5 C for 3/4 - 1 hour. Following this time the supernatant was removed.

A portion of the supernatant was taken through the digestion procedure using the N-acetyl-L-Cysteine-NaOH procedure (Vestal, 1976). See Chapter 4 for a full description. When the control soil was used it was homogenized in the Waring blender. Then the sample was digested with N-acetyl-L-Cysteine-NaOH (Vestal, 1976). The control soil sample was treated in the same fashion as the concentrated, digested leachate samples. Two preparations of the N-acetyl-L-Cysteine-NaOH solutions were made, one producing a final concentration of 4% and another producing 2%. These dilutions were designed to determine the optimum NaOH digesting concentrations.

Mycobacterium preparations from both the concentration process by the diatomaceous filters and the digestion process were used in both the undiluted state and also diluted 1:10 (with the pH 6.8 phosphate buffer) to inoculate both the Lowenstein-Jensen slants and the Middlebrook 7H10 agar plates. In the slants two drops of the undiluted, and the diluted 1:10 inocula were added. These were incubated

at 37 C for three to four weeks, up to two months. The plates listed above were inoculated by adding both dilutions to the plates in 0.1 ml amounts using the spread plate technique. The plates were kept upright for one day in the incubator stacked within plastic bags. At the end of that time, they were inverted for the remainder of their incubation period. They were checked weekly. They were kept in the incubator also up to two months.

4. Enhancing Salmonella Isolation

a. Preplating Incubation

Bacteria and leachate mixtures added to phosphate buffer or brain heart infusion broth were incubated at 37 C for 1 to 18 hr (preincubation). Then the test mixtures were plated on brain heart infusion agar and incubated at 37 C for 48 hr.

b. the Addition of Lactose or Lauryl Sulfate Broth

The procedures for the data presented in Table 53 are presented in the table. The procedures for Figure 26 will be outlined here. Four sets of data were determined and the tube contents were: phosphate buffer, 9.0 ml, inoculum, 1.0 ml; Tube 2, leachate, 9.0 ml, and inoculum, 1.0 ml; Tube 3, leachate, 8.0 ml, lauryl sulfate, 1.0 ml, and inoculum, 1.0 ml; and Tube 4, phosphate buffer, 8.0 ml, lauryl sulfate,

1.0 ml, and inoculum, 1.0 ml. These results were incubated at 37 C for one hour and plated.

From these experiments a Salmonella isolation process medium was developed. Solid waste, soil and leachate samples

were first added to brain heart infusion broth and incubated at room temperature for two hours. Following this the broth and the sample were added to selenite F broth and was incubated at 37 C for 48 hours. The growth was then examined for Salmonella.

5. The Concentration of Leachate to Aid Clostridium Isolation

Both the homogenized solid waste and the concentrated leachate samples were assayed for Clostridium enumeration. This involved the inoculation of Tryptone Sulfite Cycloserine (TSC) plates by the spread plate technique and the addition of four dilutions (1.0 to 10^{-3} ml portions) to the iron milk MPN tubes (five tube test). The plates were incubated in the GasPak (BBL) jar for 24 hours at 37 C. Some MPN tubes were incubated at 45 C (waterbath) for 24 hours, while the rest were incubated at 37 C for 48 hours in a GasPak (BBL) jar.

6. The Comparison of Streptococcal Enumeration Procedures

The eight procedures used for comparative purposes are shown in Table 29. The description of these quantitative tests have been described in detail in Chapter 4.

J. TEST PROCEDURES USED FOR SIMULATED LANDFILLS (LYSIMETERS)

1. Leachate Sampling Procedures

Leachate samples were taken for microbial and chemical analysis from The Boone County and the Center Hill lysimeters (Figure 13) in 1977 when these structures ranged in age from two to five years and from the University of Cincinnati lysimeters when field capacity was achieved and leachate was produced (Figure 14). Only the latter units were sampled on a routine basis. Additional information of leachate sampling procedures is presented at the beginning of this chapter (Chapter 4). Some of the samples used are listed in Appendix C.

2. Microbial Analysis of Solid Waste Used for Lysimeter Construction

Samples of the sewage sludge and the shredded hospital and municipal waste obtained from the wastes used in the University of Cincinnati lysimeter construction were placed in plastic bags and were kept at 5 C until they were processed. The microbial and chemical analyses used for the

TABLE 29. STREPTOCOCCAL ENUMERATION PROCEDURES FOR LANDFILL LEACHATE

Procedure Number	Streptococcal Isolation System	Streptococcal Recognition System
<u>MPN Tests</u>		
1	Azide Dextrose Broth —————→	Ethyl Violet Azide Broth (Standard Methods, 1976)
2	Azide Dextrose Broth —————→	Ethyl Violet Azide Broth (Standard Methods, 1976)
3	Azide Dextrose Broth —————→	plus Gram Stain for cocci Bile Esculin Agar (Black Growth)
4	Azide Dextrose Broth —————→	m-Enterococcus Agar (Maroon Growth)
5	Azide Dextrose Broth —————→	KF-Streptococcus Growth (Red Growth)
<u>Plate Counts</u>		
6	—————→	Bile Esculin Agar (Black Growth)
7	—————→	m-Enterococcus Agar (Maroon Growth)
8	—————→	KF-Streptococcal Agar (Red Growth)

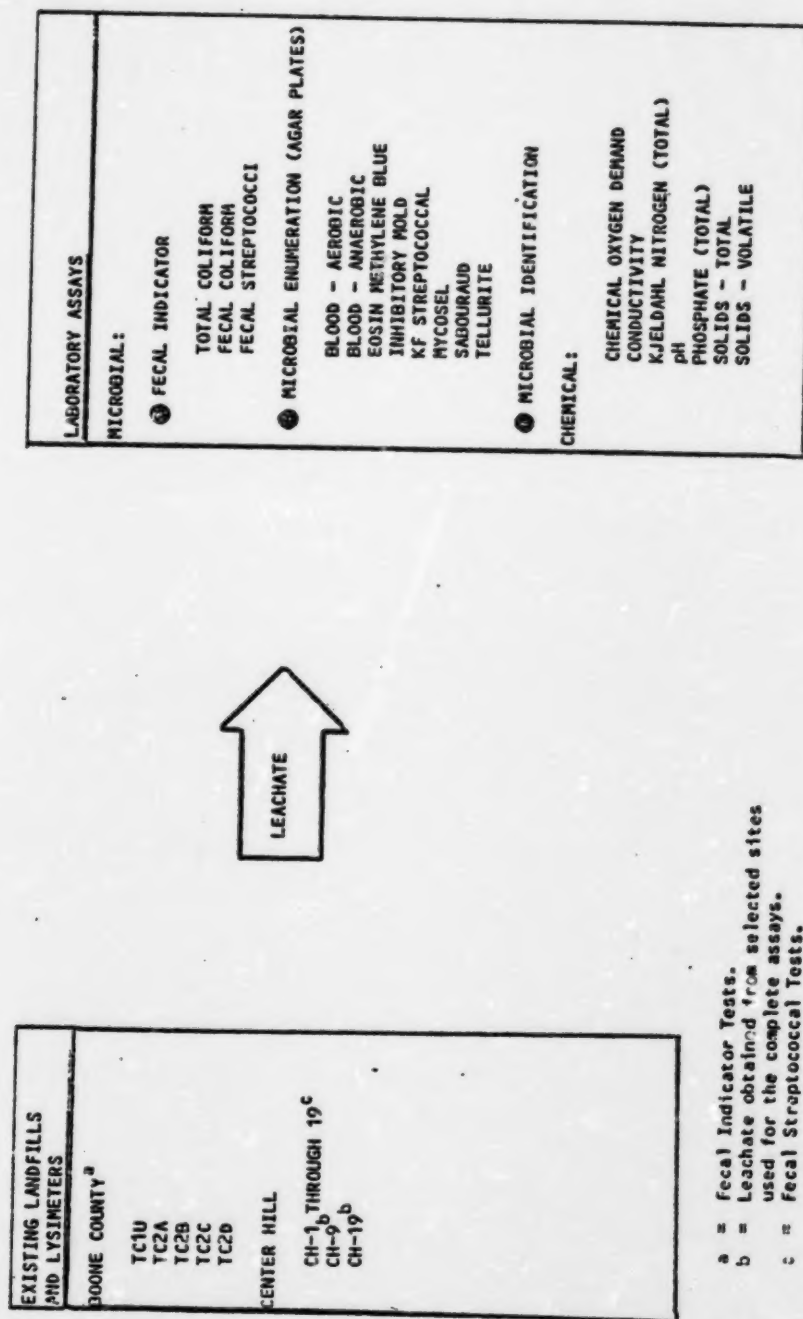


FIGURE 13. FLOW CHART OF ASSAYS MADE ON THE EXISTING LYSIMETERS

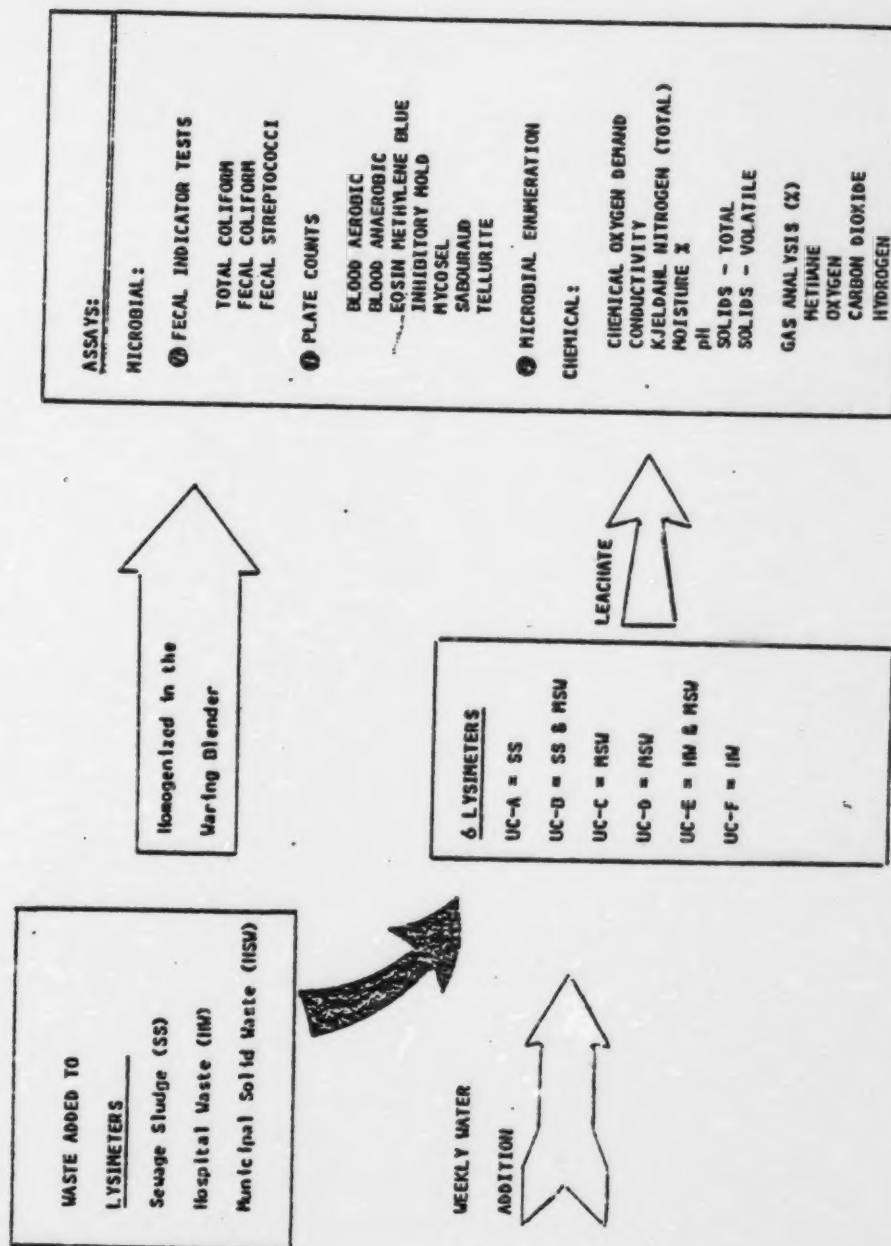


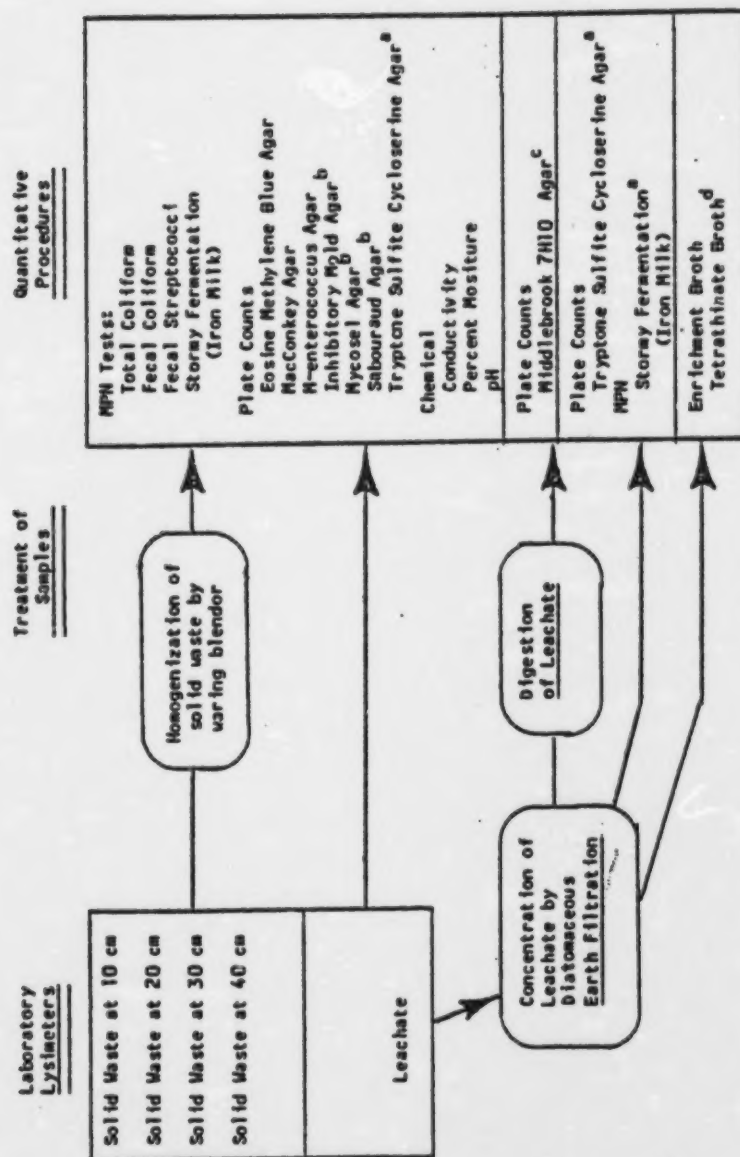
FIGURE 14. FLOW CHART OF SAMPLE TREATMENT AND ASSAY TEST

MADE ON THE SIX UNIVERSITY OF CINCINNATI LYSIMETERS

solid waste are presented in Chapter 4. This includes the solid waste homogenization process, and the fecal indicator tests, the plate counts, and the enrichment and isolation procedures. Chapter 4 also describes the microbial identification process, and the chemical analyses.

3. Final Assays on the University of Cincinnati Lysimeters

Two lysimeters, UC-D and UC-F, were opened after two years and the remaining four lysimeters, UC-A, UC-B, UC-C, and UC-E, were opened and examined after three years. The procedures are given in Figure 15. Prior to opening the lysimeters, the leachate was removed from both leachate sampling ports. Then, the lid was forced off. In all cases, the lid was so securely attached that the drum appeared to be "air-tight". The top level of the waste was examined to determine the distance that it settled during the past three years. Surface waste samples of the solid waste were not taken, but samples were taken at 10, 20, 30, and on occasion 40 cm below the top surface. The waste was removed in an area 12 inches (30 cm) in diameter and extended downward 30 to 40 cm using sterile instruments. At each level the investigator dug sideways to collect samples newly exposed to atmospheric air as anaerobic cultures were desired. As each level of the waste was reached, a kilogram of the waste sample was placed in a sterile plastic bag and



a = For *Clostridium perfringens*

b = For Fungi

c = For *Mycobacterium* sp.

d = *Salmonella* sp.

FIGURE 15. TREATMENT AND QUANTITATION OF SAMPLES

squeezed to remove all air. This sample was then kept in the refrigerator until processed, which was usually less than two hours.

4. Quantitative Procedures

For the final assay a variety of quantitative and identification tests were used which are listed in Table 30 and 31 along with the media and incubation conditions.

K. TEST PROCEDURES OF THE LANDFILL ASSAYS

1. Sampling Procedures

The leachate samples from the commercial landfill initially (1977) came from CL-4. When the bacterial counts became low in 1978 (as the source was old waste) then all sites (CL-1 through CL-5) were used. On the other hand the Boone County landfill samples initially were taken from a sampling valve (from a storage area) while the later samples at the time of the closure assays originated directly from within the landfill.

Soil (Table 32) was taken to control the commercial landfill leachate samples. Cincinnati soil was examined to isolate the characteristic Mycobacterium of this area to distinguish the natural strains from those deposited by the municipal waste. The four sites selected are listed in the table.

TABLE 30. STEPS USED IN THE QUANTITATION PROCESS AND THOSE LEADING TO MICROBIAL IDENTIFICATION.

MPN TESTS

Total Coliform

Lauryl Sulfite broth^a, Brilliant Green Bile broth^a,
Eosin Methylene Blue^b, Lauryl Sulfite broth^a

Fecal Coliform

EC broth^c

Fecal Streptococci

Azide Dextrose broth^a, Ethyl Violet Azide broth^a,
Gram Stain

Clostridium perfringens

Iron Milk^a, Egg Yolk Agar^b, Blood Plate^a,

Clostridium Identification Media

Salmonella Enrichment Broth

Tetrathionate Broth^a, Salmonella Identification Media

Chemical Parameters

Conductivity

Percent Moisture

pH

PLATE COUNTS

Eosin Methylene Blue (EMB)^a - Streak 3 times on Heart Infusion (HI)
plates, Gram-negative rod identification media.

MacConkey Agar^a - Same as EMB

m-enterococcus Agar^a - Streak 3 times on HI Agar

Middlebrook 7H10 Agar^d - Streak on agar, Mycobacterium susceptibility
tests

Inhibitory Mold Agar^e Fungal Identification Procedure

Mycosel Agar^c - Fungal Identification Procedure

Sabouraud Agar^c - Fungal Identification Procedure

TryPto^{ne} Sulfite Cycloserine Agar^b - Streak twice on Egg Yolk Agar and once
on Blood Agar Plate, then use Clostridium Identification Procedures.

a = Incubated at 37°C - 48 hours
b = Incubated at 37°C - 24 hours
c = Incubated at 44.5°C - 24 hours

d = Incubated at 37°C - weeks
e = Incubated at room temperature
for one week

TABLE 31. MICROBIAL IDENTIFICATION PROCEDURES (PART I)

Microorganisms to be Identified	Steps in the Identification Process
<u>Clostridium perfringens</u> and other clostridia	1. Isolates came from the TSC^a plates or iron milk MPN^b tubes. 2. They were streaked twice on egg yolk plates. Lecithinase and lipase production were recorded. 3. The isolate was then streaked on blood agar plate and the double zone hemolysis was recorded. Then they were Gram stained, tested for catalase production and stored in cooked meat medium with oil at room temperature after growth at 37°C. 4. The inoculum was grown at 37°C for 24 hours in cooked meat medium. One drop was placed in each tube for the following biochemical reactions^c: - Gelatin Esculin - lactose urea - motility incubated in a GasPak jar 37°C for 48 hours 5. Nagler reaction using <u>Clostridium perfringens</u> Type A and Type B antisera on egg yolk plates. Incubated in a GasPak jar at 37°C for 24 hours. 6. API-20A - GasPak jar - 48 hours

a = Tryptone sulfite cycloserine plates

b = Most-probable-number

c = Washington (1981)

TABLE 31. MICROBIAL IDENTIFICATION PROCEDURES (PART II)

Microorganisms to be Identified	Steps in the Identification Process
Gram-negative Rods	1. Taken from EMB^a, MacConkey, or XLD^b plates. 2. Streaked three times on HI^c agar plates. 3. Biochemical Reactions^d and Stains: - API-20E O/F Test^e, Glucose - Catalase Test Phenylalanine Deaminase Test - Gram Stain Triple Sugar Iron agar - MacConkey Agar Growth - Motility
<u>Mycobacteria</u>	1. Taken from Middlebrook 7H10 agar plates. 2. Acid-fast stain. 3. <u>Mycobacterium</u> susceptibility tests (Vestal, 1976)
Fungi	1. Taken from IMA^f, Mycosel, or Sabouraud Agar. 2. Examination of fungal material microscopically. 3. Fungal identification process, Koneman et al. (1978)

a = Eosin methylene blue agar

b = Xylose lysine desoxycholate agar

c = Heart infusion agar

d = Weaver et al. (1972)

Koneman et al. (1979)

Edwards & Ewing (1962)

e = Oxidation/fermentation test medium with glucose

f = Inhibitory mold agar

TABLE 31. MICROBIAL IDENTIFICATION PROCEDURES (PART III)

Microorganisms	Media and Reagents
<u>Salmonella</u> Isolations	1. Inoculated sample into: Brain Heart Infusion Broth for 2 hours, GN, Selenite F Broth, and Tetrathionate Broth for 2 days at 37°C. 2. Streaked on: Hektoen Enteric Agar Brilliant Green Agar Xylose Lysine Deoxyribose Agar Bismuth Sulfite Agar
<u>Salmonella</u> Identification ^a	1. Inoculated isolates into: Triple Sugar Iron Agar Motility Semi Solid Agar Phenylalanine Agar <u>Salmonella</u> Antiserum, A, B, Vi AP I-20E

a = Edwards & Ewing (1962)

TABLE 31. MICROBIAL IDENTIFICATION PROCEDURES (PART IV)

Test Types	Tests
<u>Streptococcal Groups</u>	
1. biochemical tests ^a	Bacitracin Sensitivity Tests Hippurate Hydrolysis Growth on Bile Esculin Growth on 6.5% NaCl
2. Streptex ^b	Agglutination Tests using Antiserum against Group A, B, C, D, F, and G
<u>Streptococcal Species</u>	
- biochemical tests ^c	Gram Stain Growth on KF Streptococcal Agar Catalase Growth on 40% Bile Growth at 10 and 45 C Starch Hydrolysis Growth in 6.5% NaCl and pH 9.6 BHI broth Growth in 0.1% Methylene Blue skim milk Reduction of tellurite and tetrazolium Fermentation of D-sorbitol, glycerol, and lactose Hydrolysis of gelatin Beta Hemolysis Litmus milk peptonization

a = Facklam et al. (1974)

b = Wellcome Research Laboratories, Beckenham, England, BR3 3BS

c = Pavlova et al. (1972) and Bordner et al. (1978)

TABLE 32. CONTROL SOIL SAMPLES TAKEN FOR MYCOBACTERIUM ISOLATION PURPOSES

Sample Number	Site	Location	Date
1.	Woods, 500 feet from Residential Area	Mt. Washington, Cincinnati, Ohio	2/3/81
2.	Farm, Vacant Field	Southeastern Indiana near Ohio River	2/4/81
3.	Flower Bed near Old Chemistry Building	University of Cincinnati, Cincinnati, Ohio	2/5/81
4.	Grass Area near Baldwin Hall	University of Cincinnati, Cincinnati, Ohio	2/6/81

The solid waste and soil samples from the Boone County landfill, and control soil near the same site, were taken at levels of 1.5 feet (0.46 meters) down to 13 feet (3.96 meters) below the ground surface (see Figures 16 and 17). The samples were placed immediately in sterile plastic bags and were squeezed to remove the air. All samples were placed in the ice chest for transportation back to the laboratory and placed in the refrigerator at 5 C until processed (within 24 hours).

2. Sample Treatment

Leachate was concentrated by diatomaceous earth filtration and both concentrated and digested leachate samples were used to isolate Clostridium and Mycobacterium, respectively. Control soil from the Cincinnati area used for Mycobacterium isolation was homogenized in the Waring blender. All of these procedures are described in the first part of Chapter 4.

3. Microbial Assays

The procedures for the streptococcal assays included eight quantitative tests, and the identification of streptococci groups and species (Table 33).

The procedures for the isolation and identification of Mycobacterium, Salmonella, and Clostridium, from the commercial landfill are listed in Table 34.

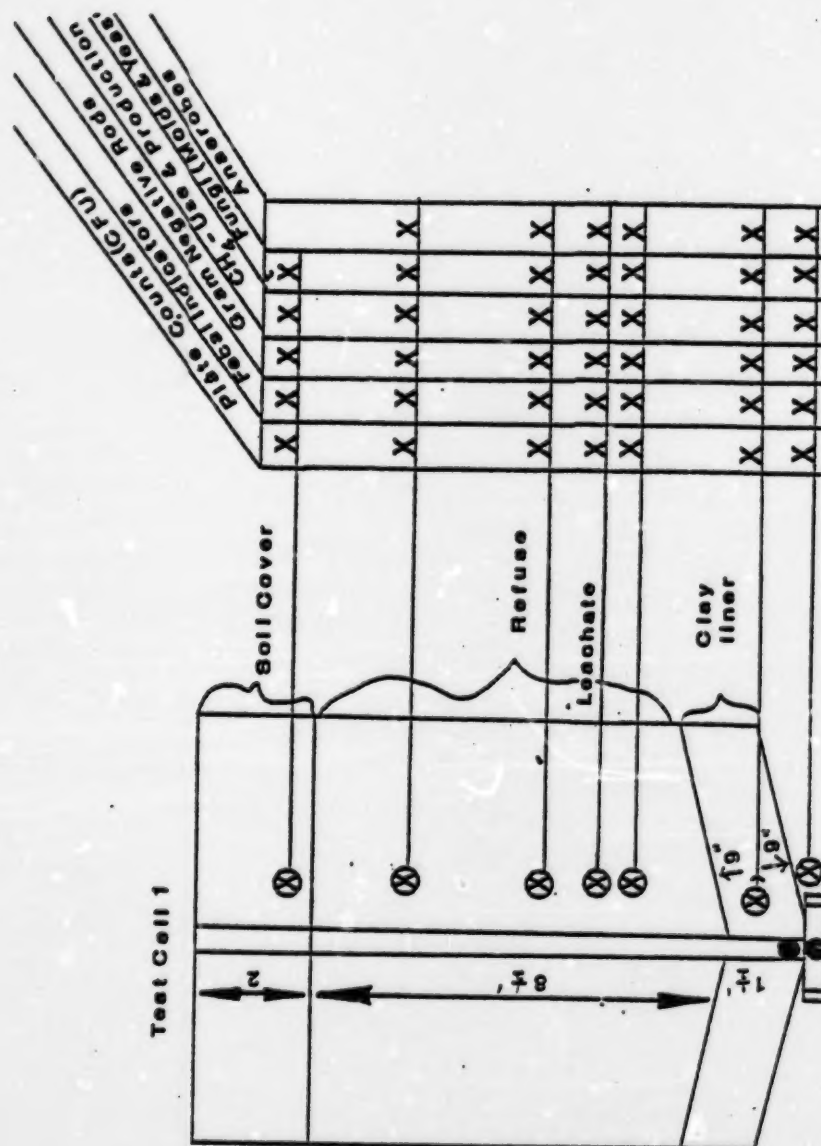


FIGURE 16. SAMPLES TAKEN FROM TC-1 AND THE TEST PROCEDURES USED

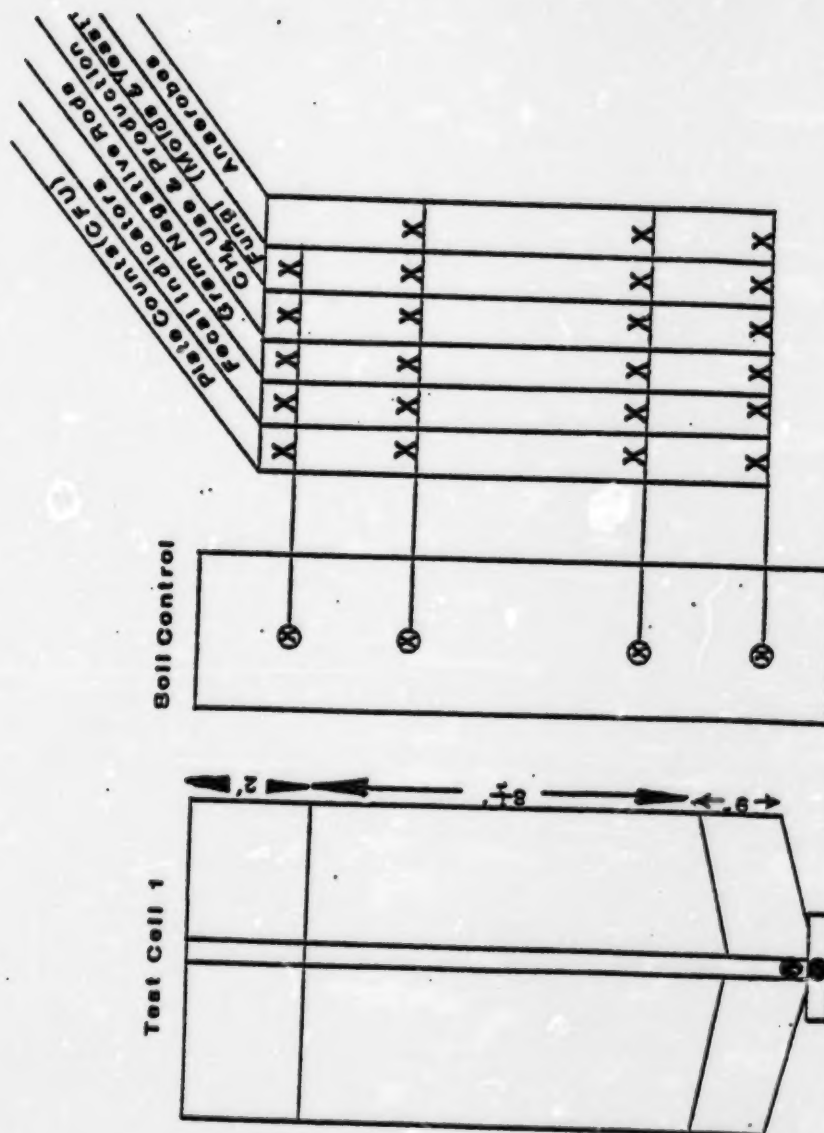


FIGURE 17. SAMPLES TAKEN FROM A SOIL CONTROL AT THE LEVELS INDICATED AND THE TEST PROCEDURES USED.

TABLE 33. PROCEDURES USED IN THE ENUMERATION AND IDENTIFICATION OF STREPTOCOCCAL ISOLATES^a

Eight Enumeration Procedures

Most-Probable-Number

Standard Methods (1976) Modified by Allen et al. (1976)

Standard Methods (1976) Plus Gram Staining for Cocci

Azide dextrose Broth Plus Bile Esculin Agar

Azide dextrose Broth Plus m-Enterococcus Agar

Azide dextrose Broth Plus KF-Streptococcal Agar

Plate Counts

Bile Esculin Agar

m-Enterococcus Agar

KF-Streptococcal Agar

Identification Methods

Grouping Procedures

Facklam et al. (1974)

Streptex (Wellcome Laboratories)

Biochemical Species Procedures

Pavlova et al. (1972) Bordner et al. (1978)

^a = From commercial landfill sites, CL-1 and CL-2.

TABLE 34. MEDIA AND PROCEDURES USED IN THE ENUMERATION AND IDENTIFICATION OF SALMONELLA, CLOSTRIDIUM, AND MYCOBACTERIUM

Media and Procedures used	References
Fecal Indicator MPT tests ^a	<u>Standard Methods</u> (1976), Allen et al. (1976)
<u>Salmonella</u>	
Broths	
Double Strength Brain Heart Infusion	
Selenite F	
Tetrathionate	
Dulcitol-Selenite	
Agar	
Xylose lysine deoxycholate agar	
Brilliant Green Bile	
Bismuth Sulfite	
Hektoen Enteric	
<u>Salmonella</u> Identification	
API-206	Analytab Products, Inc. Plainview, NY
Difco Antisera	Edwards & Ewing (1962)
Biochemical Tests	Weaver et al. (1972) Koneman et al. (1979)
<u>Fungi</u>	
Sabouraud Agar	Koneman et al. (1978)
<u>Clostridium</u>	
Iron Milk Medium	
Cooked Meat Medium	
Blood Agar Plates	
GasPak (BBL) Jar, used to incubate	Koneman et al. (1979)
all media	
<u>Mycobacterium</u>	
Isolation:	
Lowenstein-Jensen Medium	
Middlebrook 7H10 Agar	Lennette et al. (1974)
Susceptibility Tests (Vestal, 1976)	
Middlebrook 7H10 Agar	Lennette et al. (1974)

^a = including the Gram Stain of all Positive Fecal Streptococcal tests.

For the Mycobacterium isolation and enumeration the leachate was concentrated using diatomaceous earth filtration. The sample was then digested and plated. Strains were separated by the susceptibility test. Control soil (Table 32) was treated as solid waste in that it was homogenized in the Waring blender, digested and plated.

The Salmonella isolations included enrichment procedures to decrease the microbial stress on differential and inhibitory media. For the main part this involved the addition of 50 ml of leachate to 50 ml of double strength brain heart infusion broth (BHI) followed by an incubation at 37 C for 24 hours. Following this, 50 ml of the leachate-BHI broth mixture was inoculated into double strength selenite F and tetrathionate, and dulcitol-selenite broths. All cultures were incubated for 96 hours at 37 C. Two subcultures daily were streaked on xylose lysine desoxycholate, brilliant green bile, Bismuth sulfite and Hektoen enteric agars. Isolates were further streaked on MacConkey agar plates to obtain pure cultures.

The generalized anaerobe procedure were derived from Koneman et al., (1979). A portion of the concentrated leachate filter material was inoculated into a boiled and cooled iron milk tube. It was then placed in an anaerobic jar for a week. The appearance of digestion and gas formation in the iron milk indicated the growth of a Clostridium.

The microbe was gram stained; streaked onto blood agar plates and incubated in an anaerobic jar. If double zone hemolysis appeared upon the plate, the microorganism was identified as Clostridium perfringens.

The procedures for the closure of the Boone County landfill are listed in Table 35. The leachate and solid waste were homogenized in a Waring blender and enumerated. The pH, conductivity, and percent moisture were included as a means of comparing the characteristics of the soil and solid waste. Finally, the Salmonella enrichment procedure described above with the Mycobacterium cultivation process was also included in this experiment.

TABLE 35. PROCEDURES USED IN THE CLOSURE OF THE BOONE COUNTY LANDFILL

Media and Procedures used	References
Fecal Indicator Tests	
Most-Probable-Number Tests	
Total Coliforms Modified	<u>Standard Methods</u> (1976) Allen et al. (1976)
Fecal Coliforms Modified	<u>Standard Methods</u> (1976) Allen et al. (1976)
Fecal Streptococcus, Modified by Gram Staining for Cocci	
Plate Counts	
Fecal Streptococcus m-enterococcus agar	Dudley et al. (1980)
<u>Salmonella</u> and Related Gram-Negative Rods	
Enrichment Broths	
Selenite F GN	
Isolation Agars	
Xylose Lysine Deoxycholate Hektoen Enteric	
Identification	
Antisera (Difco) Biochemical Tests	Edwards & Ewing (1962) Weaver et al. (1972) Koneman et al. (1979)
Chemical Procedures	
pH Conductivity Percent Moisture	

CHAPTER V

RESULTS: THE DEVELOPMENT OF METHODS

A. INTRODUCTION

Initially the methods selected for this work were derived from those in current use such as the Standard Methods (1976), and Lennette et al., (1974). However, the methods chosen did not always work with efficiency but were on occasion nonspecific and imprecise. Some of the problems encountered with the methods included the following:

1. the growth of fewer microbial colonial types from leachate following freezing of leachate samples,
2. problems with pure culture isolation,
3. needed a stock culture storage system,
4. no growth in high concentrations of leachate, but growth appeared in more dilute portions,
5. nonspecific tests,
6. no isolation of pathogens from samples containing sewage sludge, and other wastes,
7. variations in concentrations of streptococci by two or more methods,
8. the meaning of MPN tests as compared to plate counts,
9. bacterial stress.

Short term studies were done to analyze these methods and to improve their performance. However, not all of these problems were solved, but the analysis made in procedure strongly suggests that further improvements would greatly increase the pathogen and fecal indicator identifications.

B. STORAGE OF LEACHATE

As it was necessary to analyze many leachate samples to determine the significance of microorganisms in the landfills, we looked for some efficient way to group the leachate assays and perhaps do many at the same time. This would involve storing the leachate for weeks or months. As the storage of these samples in the refrigerator for extended periods of time could precipitate the protein, evaporate the sample or make other changes, and thus affect the microbial counts, it was decided to freeze the samples. However, the effect of freezing on the leachate was also unknown (Cooper et al., 1974). Before a large number of leachate samples were frozen, a short leachate freezing assay was made. This was done by comparing the plate counts and the number of colony types (as determined by appearance and by the Gram reaction) present in leachate samples before and after freezing. The results are presented in Table 36.

TABLE 36. THE DECREASED NUMBER OF MICROBIAL COLONY TYPES IN LEACHATE FROZEN SAMPLES COMPARED TO THOSE NOT FROZEN

Leachate ^a Source	Plate Counts ^b CFU/ml	Number of Colony Types ^c					Loss Total %
		Yeast	Gram- Negative Rods	Gram-Positive			
				Cocci	Rods		
<u>CH-9</u>							
Not Frozen	1.9X10 ³	3	3	1	6	13	
Frozen ^d	1.0X10 ²	0	1	0	1	2	85
<u>CH-19</u>							
Not Frozen	2.4X10 ³	0	2	1	11	14	
Frozen	3.6X10 ²	0	2	0	3	5	64
<u>TC2D</u>							
Not Frozen	6.2X10 ³	0	1	1	4	6	
Frozen	1.9X10 ³	0	0	0	6	6	0
<u>Commercial Landfill</u>							
Not Frozen	1.3X10 ²	0	3	1	6	10	
Frozen	4.0X10 ¹	0	1	0	4	5	50

a = All leachate samples were taken on 11/15/77 except for the Commercial landfill, which was taken two days later.

b = Prepared using BHI agar, incubated at 37 C for 48 hours.

c = Determined by Gram-stain.

d = Leachate frozen at -80°C for one week.

The results show that freezing decreased the plate counts in most instances by one log. More important, some colony types were killed by the freezing process. In comparing the colony types, it was found that from 50 to 85 percent of the colony types were destroyed by the freezing process in three-quarters of the samples examined. Thus, freezing samples was not a desired procedure, and the use of fresh leachate was preferable to freezing. Consequently, for the completion of the various projects in this research, fresh leachate, not frozen, was evaluated. When the fresh leachate was brought to the laboratory it was kept in the refrigerator at 5 C and processed within 24 hours, and not more than 48 hours.

C. THE SELECTION OF PURE CULTURES

The isolation of microbial strains to produce pure cultures is an essential step for identifying microorganisms. However, in practice it was difficult to carry out. Often it was impossible to separate the spreaders from the slow growing bacteria on certain media. In addition contaminants were found in these cultures already isolated and assumed to be pure. Thus, the following procedures were developed because simple streaking procedures often did not bring about pure culture isolations.

1. Clostridium perfringens

C. perfringens growing on blood agar plates following anaerobic incubation at 37 C for 24 to 48 hr usually were contaminated with spreaders. However, by first streaking the C. perfringens growth on egg yolk plates twice in succession and incubating the plates at 37 C for 24 hrs followed by a third streak plate using blood agar and incubated under the same conditions, C. perfringens colonies could be obtained in pure culture without the presence of the spreader.

2. Gram-Negative Rods

Gram-negative rods were streaked and isolated three or more times on brain heart infusion agar at 37 C and once at 25 C. This process often uncovered a second culture accompanying the first "isolate". When the second culture was another Gram-negative rod, it was frequently difficult to detect. This second culture undoubtedly was stressed for it grew at a slower rate than the first culture. With time it probably repaired itself on the enriched brain heart infusion agar and grew at room temperature to produce visible colonial growth. Thus, environmental Gram-negative rods may have to be reisolated prior to use, or at any rate, undergo a week-long incubation at room temperature (25 C) to give the stressed contaminant time to grow. From our

experiences with 40 cultures stored at room temperature on brain heart infusion agar slants, eighteen previously isolated strains contained mixed cutures.

3. Streptococci

Frequently pure cultures of streptococci could not be obtained by streaking colonial growth on brain heart infusion agar followed by an incubation period for 48 hr at 37 C as the cultures were contaminated with diptheroids. However, by streaking streptococcal growth on the various differential agars used in the identification tests, these diptheroids were removed. The following agars were streaked in succession and incubated at 37 C for the following hours: brain heart infusion agar (48 hr); KF Streptococcal agar (48 hr); TTC (2,3,5-Triphenyl tetrazolium chloride) agar (48 hr); and tellurite agar (120 hr). Many streptococcal strains were isolated with ease but others were seemingly permanently attached to diptheroids (50%). It was only by following the above scheme that the streptococci were finally isolated free from the diptheroids.

D. CULTURE STORAGE

Initially all stock cultures were kept in skim milk at -80C. However, when the Salmonella typhi lost its Vi antigen following a two year storage under these conditions, then the storage procedures were changed. Accordingly,

cultures were inoculated into heart infusion agar deep slants (8 ml in a 13 X 100 mm screw capped pyrex tube). Moreover, it was necessary to screw the caps tightly to the mouth of the tube as the drying of the agar often killed the culture. After incubation and growth these tubes were stored at room temperature (22-25 C). Often these cultures lasted over two years under these conditions.

E. TOXICITY AND NONSPECIFICITY IN MPN TESTS

The fecal streptococcal MPN test (Standard Methods, 1976) was the standard for enumeration levels of leachate in this study. Positive levels indicated that streptococci were present in the leachate. Thus the leachate was placed into cooked meat medium and incubated at 37 C for 10 days to grow streptococcal cultures. Then the tubes were Gram stained at the end of two and ten days. The results are shown in Table 37. Streptococci were present in only one cooked meat tube at the end of two days, while eight tubes showed growth at the end of ten days. Of the nine positive cultures, three came from leachate that had <3 MPN/100 ml of streptococci while six came from leachate containing 110 to 2300 MPN/100 ml of leachate. Of the negative tubes, five came from leachate that had streptococci ranging from 230 to 1300 MPN/100 ml of leachate. These were probably stressed bacteria which required a longer than usual incubation time

TABLE 37. THE CULTIVATION OF STREPTOCOCCI FROM LEACHATE IN COOKED MEAT MEDIUM^a

Leachate ^b Source Test Cell Number	pH of Leachates	Fecal ^c Streptococci MPN/100 ml	Streptococcal Growth in Cooked Meat Medium (37 C)	
			After 48 hours	After 10 days
CH-1	5.29	1300	- ^d	-
CH-2	5.13	1700	-	+ ^e
CH-3	5.07	< 2	-	+
CH-4	5.17	2	-	-
CH-5	4.70	800	-	-
CH-6	5.32	280	-	-
CH-7	5.29	500	-	+
CH-8	5.35	700	-	+
CH-9	5.54	200	-	+
CH-10	5.41	< 2	-	+
CH-11	5.18	< 2	-	-
CH-12	6.09	2400	-	-
CH-13	5.45	110	-	+
CH-14	5.30	2300	-	+
CH-15	5.10	< 2	-	-
CH-16	5.23	< 2	-	-
CH-17	5.41	< 2	+	-
CH-18	5.16	230	-	-
CH-19	5.25	230	-	-

a = One tenth milliliter of leachate was added to each tube.

b = Center Hill lysimeters.

c = Standard Methods (1976)

d = No Gram-positive cocci as seen on bacterial smear.

e = Gram-positive cocci seen on bacterial smears.

for growth. Perhaps they were also stressed by the low pH, which was between 4.7 and 6.09. Nevertheless there was a concern over the use of this MPN test to determine streptococcal concentrations.

A further examination of these leachates may be seen in Table 38. here the number of positive tubes per dilution is given. Apparently the lowest two dilutions, the 10 ml and the 1.0 ml tubes, gave a lower number of positive results than did the 10^{-1} ml and 10^{-2} tubes. This occurred in eight out of eleven leachate samples. In most instances all five tubes of these dilutions were inhibited. This occurred in seven out of eight leachate samples, indicating that the leachate was toxic and that it inhibited the positive reactions. As the streptococcus was certainly present in the leachate prior to contact with the ethyl violet azide broth, perhaps the inhibiting components in the broth prevented the coccal growth.

TABLE 38. AN EXAMINATION OF LEACHATES^a FROM CENTER HILL TEST CELLS FOR THE PRESENCE OF FECAL STREPTOCOCCI

Leachate ^a Source Test Cell Number	Fecal Streptococci ^b	
	Positive Tubes	MPN/100 ml
CH-1	(5-5-4-0)	1300
CH-2	(5-5-4-1)	1700
CH-3	(0-0-0-0)	< 2
CH-4	(0-1-0-0)	2
CH-5	(0-0-3-0)	800
CH-6	(5-4-3-0)	280
CH-7	(0-0-2-0)	500
CH-8	(0-0-5-0)	700
CH-9	(0-0-1-0)	200
CH-10	(0-0-0-0)	< 2
CH-11	(0-0-0-0)	< 2
CH-12	(0-5-5-0)	2400
CH-13	(0-3-1-0)	110
CH-14	(1-5-5-0)	2300
CH-15	(0-0-0-0)	< 2
CH-16	(0-0-0-0)	< 2
CH-17	(0-0-0-0)	< 2
CH-18	(X ^c -5-0-0)	230
CH-19	(X-5-0-0)	230

a = Taken on 3/7/77 c = No tube at the 10 ml concentration

b = Standard Methods (1976) test with leachate concentrations of 10.0 ml through 10² ml.

1. Gram Stain Used to Improve MPN Specificity

A further examination of the MPN test described in Table 38 is seen in Table 39. Since the streptococci did not grow from the positive tubes as shown in Table 38, all ethyl violet azide tubes were Gram stained and examined for Gram-positive cocci. These results are shown in Table 39. Here for comparison are the number of positive tubes as determined by the Standard Methods (1976) MPN test and the number of positive tubes as determined by the Standard Methods (1976) test with the Gram stain test for cocci. The results show that a considerable number of the positive tubes (those containing purple growth) contained no Gram-positive cocci, but Gram-positive rods, instead. Thus, Gram-positive rods in addition to the cocci brought about the purple growth, the positive test indicator. Furthermore, the number of positive tubes indicated that there are more positive tubes with the Standard Methods (1976) MPN test than with the Standard Methods (1976) MPN test with the Gram stain test for cocci. Thus, out of a total of 13 lysimeter samples with fecal streptococcal levels, six contained streptococci as determined by the adapted method of the Standard Methods (1976) with the Gram-stain. Furthermore, overall there were 88 positive tubes which were determined by the Standard Methods test without the Gram stain as compared to 25 positive tubes by the

TABLE 39. STREPTOCOCCAL LEVELS OF CENTER HILL LYSIMETER LEACHATES

Leachate ^a Source	Fecal Streptococci MPN/100 ml, Examined by:			
	Standard Methods (1978)		Standard Methods + Gram Stain	
	# Positive ^{b,d}	Levels	# Positive ^{b,c,d}	Levels
CH-1	(5-5-4-0) =	1300	(0-1-0-0) =	20
CH-2	(5-2-4-1) =	1700	(0-0-3-1) =	1100
CH-3	(0-0-0-0) =	< 2	(0-0-0-0) =	< 2
CH-4	(0-1-0-0) =	2	(0-0-0-0) =	< 2
CH-5	(0-0-3-0) =	800	(0-0-0-0) =	< 2
CH-6	(5-4-3-0) =	280	(0-0-0-0) =	< 2
CH-7	(0-0-2-0) =	500	(0-0-0-0) =	< 2
CH-8	(0-0-5-0) =	700	(0-0-0-0) =	< 2
CH-9	(0-0-1-0) =	200	(0-0-0-0) =	< 2
CH-10	(0-0-0-0) =	< 2	(0-0-0-0) =	< 2
CH-11	(0-0-0-0) =	< 2	(0-0-0-0) =	< 2
CH-12	(0-5-5-0) =	2400	(0-2-4-0) =	1300
CH-13	(0-3-1-0) =	110	(0-0-0-0) =	< 2
CH-14	(1-5-5-0) =	2300	(0-1-3-0) =	800
CH-15	(0-0-0-0) =	< 2	(0-0-0-0) =	< 2
CH-16	(0-0-0-0) =	< 2	(0-0-0-0) =	< 2
CH-17	(0-0-0-0) =	< 2	(0-0-0-0) =	< 2
CH-18	(X ^e -5-0-0) =	230	(X-5-0-0) =	230
CH-19	(X-5-0-0) =	230	(X-5-0-0) =	230

a = Date of sample = 3/7/77.

b = With 10 ml, 1.0 ml, 10⁻¹ and 10⁻² ml of leachate.

c = Contain Gram positive cocci.

d = When 10 or 1 ml samples gave no or reduced positive tubes than higher dilutions, presumably due to leachate toxicity which was diluted out, the results of these higher dilutions were used for the MPN calculations.

e = No tube at the 10 ml concentration.

Standard Methods test with the Gram stain. Thus, less than 50% of the leachate samples were positive by the Standard Methods (1976) and less than 33% of the tubes were positive by the Standard Methods with the Gram stain procedure. By comparing the results from a variety of sources, it was found that these nonspecific positive tests occurred generally in great numbers in old lysimeters such as the two year old Center Hill lysimeters, and to a lesser degree in the landfill and new lysimeter assays.

2. The Removal of Toxicity by Leachate Dilution

As reported above, ethyl violet azide MPN tubes containing 10 ml and 1.0 ml of leachate were often negative while more leachate diluted tubes such as those containing 10^{-1} ml and 10^{-2} ml were positive. This suggested that dilutions of the leachate might bring about a decrease in the toxicity for the streptococci. To test this conclusion a series of leachate dilutions were placed in contact with a constant concentration of test bacteria. These results are shown in Table 40. These results show that the undiluted leachate had low concentrations of bacteria (10^2 CFU/ml) while higher dilutions in many cases contained increased bacterial numbers (10^3 to 10^5 CFU/ml) indicating that multiplication had occurred once the leachate toxic agent was diluted out.

TABLE 40. THE DILUTION OF LEACHATE^a PRIOR TO PLATING TO INCREASE THE SURVIVAL OF BACTERIA

Experiment Date	Source of Leachate & Sample Date	Hours of Incubation at 37 C	Plate Counts (CFU/ml) of Bacteria		
			Undiluted	Leachate Dilutions 10 ⁻¹	10 ⁻²
6/5/78	Commercial Landfill (6/8/78)	0	2.4X10 ²	10	5
		1	1.05X10 ²	5.5X10 ²	10
		3	2.4X10 ²	2.0X10 ³	5
		6	6.6X10 ²	9.6X10 ³	1.3X10 ³
6/8/78	CH-19 (6/6/78)	0	TTC ^b	5.3X10 ³	20
		1	2.95X10 ²	1.76X10 ⁴	1.1X10 ⁴
		3	2.1X10 ²	1.9X10 ⁴	1.3X10 ⁴
		6	1.5X10 ²	4.6X10 ⁴	1.3X10 ⁵

a = Leachate in 1.0 ml amounts were added to 9.0 ml of brain heart infusion (BHI) broth. Further dilutions were made with BHI broth, and all these were incubated at 37 C for 1 through 6 hours. Then samples were diluted with phosphate buffer and plated by the spread plate technique using brain heart infusion agar which was incubated at 37 C for 48 hours.

b = Too many to count

3. The Demonstration of Leachate Toxicity with Plate Counts

The toxic reactions suggested to us that perhaps the leachate would inhibit the pathogens to such an extent that the bacteria would not grow on the differential plate media. This possibility was tested and the results are presented in Figure 18. Here the microbial levels of pathogens and a fecal indicator, following contact with phosphate buffer and leachate for one-half an hour are compared. The leachate decreased the levels of three types of microorganisms by two logs and a fourth by four logs. The leachate studied was that from the Center Hill lysimeter CH-19, 11/17/77 (two years old). Similar results were found using a variety of leachates (see Table 41) in which the bacterial counts also decreased after a one-half hour and a 24 hour contact with the leachate, the streptococci were more resistant than the Salmonella to all four leachates. In addition, the leachate from the landfills TC-1 was less toxic than the leachate from the lysimeter and TC2C to both the Salmonella and the Streptococcus.

The results of the coliform MPN tests of leachate, described above in Tables 37, 38, and 39, from the sewage sludge lysimeter showed that in some instances the total coliforms remained steady or increased in numbers but the fecal coliforms had apparently died out as the concentra-

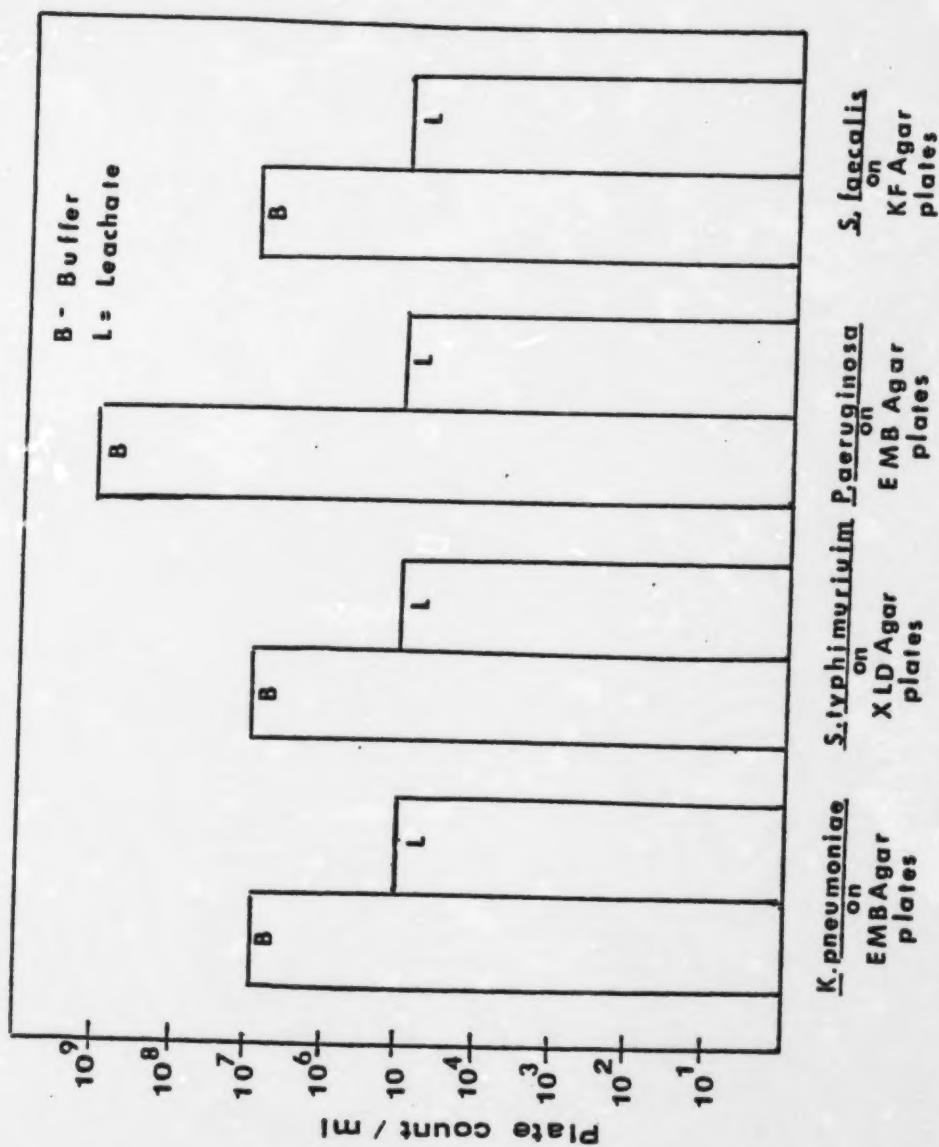


Figure 18. THE INHIBITION OF LANDFILL LEACHATES FOR KLEBSIELLA PNEUMONIAE, SALMONELLA TYPHIMURIUM, PSEUDOMONAS AERUGINOSA, AND STREPTOCOCCUS FAECALIS.

TABLE 41. THE INHIBITION OF MICROORGANISMS ADDED TO LANDFILL (LYSIMETER) LEACHATE.

TABLE 41. THE INHIBITION OF MICROORGANISMS

Bacteria	Test Bacteria	CFU/ml	Leachate ^c Source	Bacteria and Leachate were plated on:	The survival (CFU/ml) of test bacteria added to leachate and plated after a contact time (at 37 C) of:	
					30 minutes	24 hours
Center Hill Leachate Experiments (01/05/78)						
<u>Salmonella typhimurium</u>		5.8x10 ²	CH-9	XLD ^c	< 10	< 10
(Manning)		5.8x10 ²	CH-19	XLD	< 10	< 10
<u>Streptococcus faecalis</u>		3.9x10 ²	CH-9	KF ^d	1.5x10 ²	< 10
ATCC #19433		3.9x10 ²	CH-19	KF	4x10 ¹	< 10
Boone County Landfill Experiments (01/25/78)						
<u>Salmonella typhimurium</u>		1.2x10 ³	TC-10	XLD	2.2x10 ²	ND ^e
(Manning)		1.2x10 ³	TC-2C	XLD	< 10	ND
<u>Streptococcus faecalis</u>		6.6x10 ³	TC-10	KF	1.3x10 ³	ND
ATCC #19433		6.6x10 ³	TC-2C	KF	3x10 ²	ND

a = Leachate collected on 01/05/78 from Center Hill Lysimeters CH-9 and CH-19.

b = Leachate collected on 01/24/78 from the Boone County Landfill TC-10 and Lysimeter TC-2C.

c = Inoculum in leachate = 1:100 dilution.

d = xylose lysine desoxycholate agar, incubated 37 C for 24 hr.

e = KF Streptococcal agar, incubated at 37 C for 48 hr.

a = Not determined.

tions remained at < 2/100 ml. Before the utilization of the Allen et al., (1976) technique on (lysimeters aged four through thirteen weeks) the total coliform MPN counts had been less than 20/100 ml. But at week thirteen when the Allen et al., (1976) technique was utilized, the counts went up to 20,220,,130,113,40,11, and 11 MPN/100 ml on weeks: 13; 15; 17; 19; 22; 27; and 31, respectively. These results indicate that the method increased the concentration levels. Thus, probably these stress reducing techniques increased the growth of fecal indicators and pathogens by allowing stressed bacteria to repair and multiply.

F. ENHANCING SALMONELLA ISOLATION
(Bacterial Stress Procedures)

The Salmonella and Shigella enrichment broth inoculations were made by transferring 5 ml of leachate into single strength broth or 50 ml of leachate into double strength tetrathionate, selenite F, and GN broths. Following their incubation at 37 C they were streaked three to four times within a period of 24 to 48 hours on xylose lysine desoxycholate (XLD) agar plates. The results were poor, i.e., after many leachate inoculations only one Salmonella was isolated by this procedure. To increase the Salmonella growth, several methods were investigated.

1. Preplating Incubation

Other investigators have recognized that bacteria from the environment probably were stressed and have used stress-reducing procedures to increase the counts (Rose et al., 1975; and Ray et al., 1972b). Such procedures allowed the bacteria to repair and to grow. One attempt to use this process was the preplating incubation. The results are shown in Table 42, in which the leachate bacteria were incubated for several hours at 37 C in phosphate buffer or in brain heart infusion broth. The phosphate buffer offering no nutrients nevertheless brought about an increase in microbial growth by one log within three hours while the brain heart infusion broth increased the counts to the same degree after only one hour. Thereafter the counts did not increase appreciably until after six hours. This initial increase in counts probably was due to the recovery of stressed bacteria as suggested by Ray et al., (1972b).

2. The Addition of Lactose or Lauryl Sulfate Broth

The work of Thompson et al., (1977) suggested the use of lactose as a growth enhancer. They incubated environmental samples in 1% lactose broth for 24 hours prior to incubation in tetrathionate broth to increase the Salmonella isolations. In carrying out this work, lactose or lauryl sulfate was added to leachate to enhance the

TABLE 42. THE USE OF PREPLATING INCUBATION TO IMPROVE THE SURVIVAL OF LEACHATE BACTERIA

Incubation of Leachate ^a Prior to Plating (hr)	Plate Counts ^b (CFU/ml) of Leachate. Diluted for Plating with:	
	Phosphate Buffer	BHI ^c Broth
0	4.0X10 ³	4.0X10 ³
1	3.4X10 ³	2.2X10 ⁴
3	2.0X10 ⁴	7X10 ⁴
6	3.0X10 ⁴	8X10 ⁴
18	1.0X10 ³	1X10 ⁹

a = Taken from CH-19 on 05/22/78, experiment run on 05/22/78. Leachate, 1.0 ml was added to 9.0 ml of phosphate buffer or brain heart infusion (BHI) broth and incubated at 37 C for 1 through 18 hours and then plated.

b = Plated in brain heart infusion agar.

c = Brain heart infusion

growth of Salmonella typhimurium or Streptococcus faecalis. The results are shown in Table 43. The Salmonella growth was not enhanced by the lauryl sulfate. The Streptococcus on the other hand did increase in numbers in the presence of 1 % lactose. Of interest also are the results of tube 3 comparing the survival of both bacteria to the presence of leachate. The Salmonella was killed after a one-half an hour contact while the Streptococcus was only slightly decreased. This suggested the resistance of the latter microorganism to leachate.

An additional study was made to determine the effect of lauryl sulfate on decreasing the inhibitory action of leachate for Streptococcus faecalis. The results are presented in Figure 19. Here the graph shows that the leachate caused the $10^7/\text{ml}$ count to drop to less than $10^5/\text{ml}$, while the lauryl sulfate increased the count to at least one or more logs up to $10^6/\text{ml}$.

By considering these results and others to produce a Salmonella growth medium, a combination of methods was devised which included preincubation and lactose addition. For this procedure a 1% lactose solution was prepared in a mixture of 50:50 using leachate and brain heart infusion broth. This mixture was incubated at 37 C for two hours. Then 50 ml was added to a double strength selenite broth

TABLE 43. THE SURVIVAL OF BACTERIA IN LEACHATE MIXED WITH LAURYL SULFATE AND WITH 1% LACTOSE

Tube Number	Purpose of Test ^{ab}	Incubation Period 37°C (hrs)	The survival levels of these bacteria ^b (CFU/ml) in the test Reagents of: Inoculum, Leachate ^c , and Diluent ^b	
			<u>Salmonella typhimurium</u> ^d	<u>Streptococcus faecalis</u> ^e
1	Inoculum Control ^d	0	8.5X10 ³	1.1X10 ⁹
2	Leachate ^c Control	1/2	< 10	3.3X10 ²
3	Microbial Growth Inhibition Assay	1/2	< 10	8.5X10 ⁸
		2	< 10	ND ^f
4	Assay for Growth Enhancement by Lauryl Sulfate or 1% Lactose Solution		<u>Lauryl Sulfate</u>	<u>1% Lactose</u>
		1/2	2X10 ²	> 10 ⁹
		2	< 10	ND

a = Test Results: tube #1=Inoculum 0.1 ml, BHI broth 9.9 ml
 tube #2=Diluent 0.1 ml, leachate 9.9 ml
 tube #3=Inoculum 0.1 ml, leachate 9.9 ml
 tube #4=Inoculum 0.1 ml, leachate 8.9 ml, plus 1.0 ml of lauryl sulfate or 1% lactose solution

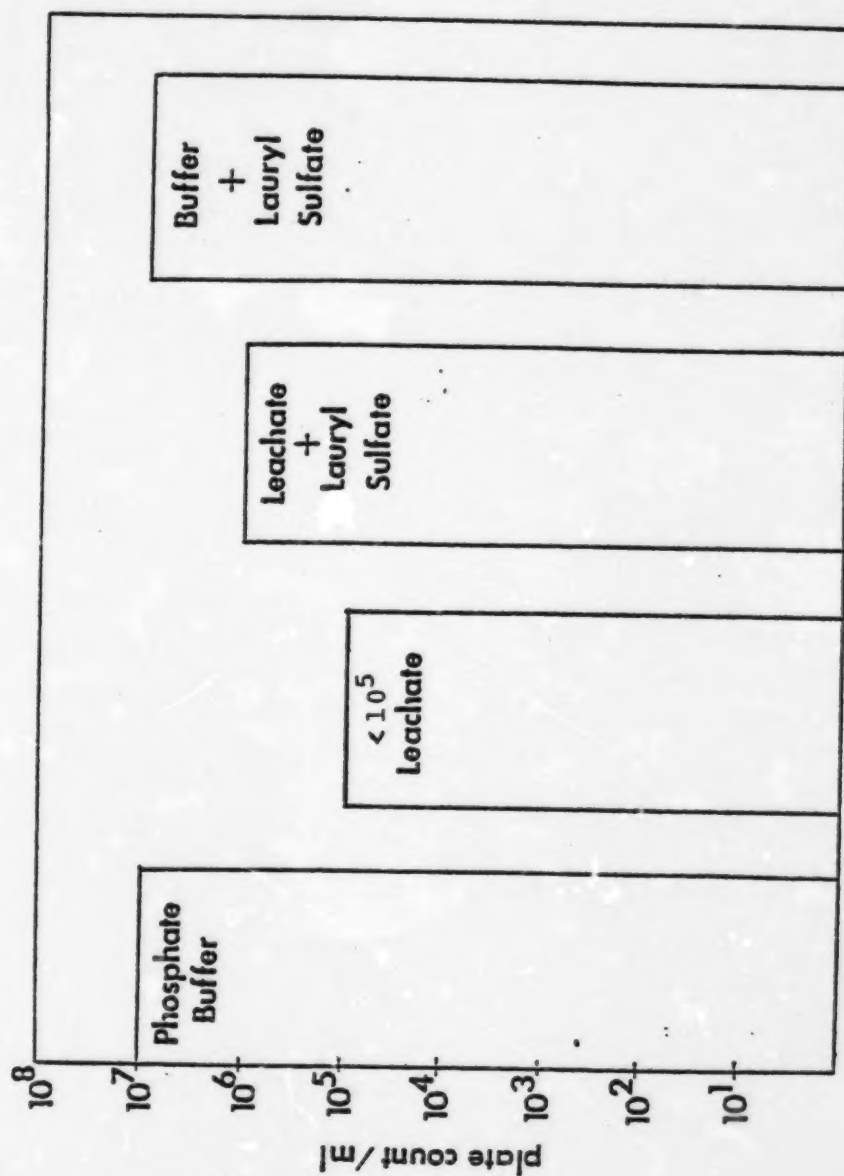
b = Diluent was phosphate buffer (Standard Methods, 1976)

c = From TC2C taken on 1/31/78 and used in the Salmonella assay.
 Leachate from TC1U taken on 2/7/78 was used in the Streptococcus analysis; fecal Streptococcus MPN/100 ml=200.

d = Plated on XLD agar, and incubated at 37 C for 24 hr.

e = Plated on KF agar, and incubated at 37 C for 48 hr.

f = Not determined.



Test Leachates and Controls
on KF Streptococcal Agar Plates

FIGURE 19. THE ADDITION OF LAURYL SULFATE BROTH IN REVERSING THE INHIBITION IN STREPTOCOCCUS FAECALIS BY LANDFILL LEACHATE^a

solution. This mixture was incubated at 37 C for 48 hours. The culture was then streaked on Hektoen enteric agar for four successive times between 18 and 48 hours. The results demonstrated (not shown here) that the procedure was acceptable, i.e., four Salmonella isolates were obtained in this manner from the commercial landfill.

3. The Addition of EDTA

Lastly, the addition of EDTA (disodium ethylenediaminetetraacetate) as a possible growth enhancer of leachate microorganisms, including Salmonella, was used. The results are presented in Table 44. Five enumeration procedures were studied: blood plate counts, both aerobic and anaerobic; and three MPN procedures, namely, total coliforms, fecal coliforms and fecal streptococci (Table 44). Of the aerobic plate counts the EDTA increased the counts in five out of six leachate samples. Of the anaerobic leachate plates, the EDTA increased only three out of six samples. Finally, in leachate samples containing surviving fecal indicators, the EDTA increased both the total coliforms and the fecal streptococcal MPN levels by two out of two samples. Therefore, it would appear that the EDTA enhanced the leachate levels to some extent. Furthermore, these results suggest that more compounds of a similar make-up should be tried.

TABLE 44. THE ADDITION OF EDTA TO LYSIMETER LEACHATES TO INCREASE THE MICROBIAL COUNTS IN PLATE COUNTS AND WITH MPN PROCEDURES

Lysimeter	Plate Counts/ml Blood Agar Plates		MPN/100 ml		
	Aerobic	Anaerobic	Total Coliform	Fecal Coliform	Fecal Streptococci
A 0.1M					
EDTA ^a	1.52X10 ⁵	8.5X10 ⁴	34	< 2	< 20
NO EDTA	1.45X10 ⁵	6.2X10 ⁴	11	< 2	< 2
B 0.1M					
EDTA	5.9X10 ⁴	6.0X10 ³	4	< 2	1300
NO EDTA	4.0X10 ⁴	1.0X10 ⁴	2	< 2	790
C .01M					
EDTA	1.37X10 ⁵	3.0X10 ⁴	< 2	< 2	5.4X10 ⁴
NO EDTA	8.8X10 ⁴	3.0X10 ³	< 2	< 2	7.0X10 ³
D .01M					
EDTA	4.7X10 ⁶	1.5X10 ⁴	< 2	< 2	< 200
NO EDTA	7.6X10 ⁶	4.0X10 ⁴	< 2	< 2	< 20
E .01M					
EDTA	1.8X10 ⁵	1.6X10 ⁴	< 2	< 2	< 2000
NO EDTA	1.7X10 ⁴	3.1X10 ⁴	< 2	< 2	2X10 ³
F .01M					
EDTA	1.9X10 ⁴	6.4X10 ⁴	< 2	< 2	< 20
NO EDTA	1.4X10 ⁴	7.5X10 ⁴	< 2	< 2	4X10 ²

a = Disodium ethylenediaminetetraacetate.

However, as the increase in count was so slight it was never used in this study.

G. THE DIGESTION AND CONCENTRATION OF LEACHATE FOR
CULTURING MYCOBACTERIUM

Mycobacteria were isolated infrequently from the commercial landfill leachate. The process used had involved digesting the leachate and inoculating the resulting sample on Lowenstein-Jensen slants (Vestal, 1976). On the other hand when concentrated leachate was added to the digestion process Mycobacterium was isolated from each leachate sample. The results are presented in Table 45. This table shows that from 0.5 to 1.0 liters of leachate were filtered from each source through the diatomaceous earth filters. A comparison of two digestion NaOH concentrations demonstrated that the 2% solution was the better choice as it allowed the growth of more mycobacterial cells. In conclusion, the concentration method allowed for Mycobacterium to be isolated from each sample, suggesting that it could be used as an enumeration procedure.

H. THE CONCENTRATION OF LEACHATE TO INCREASE CLOSTRIDIUM
ISOLATION

The first indicator used to describe the appearance of Clostridium perfringens on anaerobic blood agar plates was the double zone hemolysis. However, in the early experiments no such colony types were found. The success of

TABLE 45. MYCOBACTERIA ISOLATED FROM THE COMMERCIAL
LANDFILL LEACHATE FOLLOWING CONCENTRATION BY
FILTRATION^a

	Leachate Samples	<u>Mycobacterium CFU/100 ml</u>		Liters of Leachate Filtered
		<u>NaOH Concentrations^b</u>		
		4%	2%	
06/09/80	1	2(<1) ^c	ND ^d	0.5
	2	<1 (2)	ND	0.5
	3	32 (20)	ND	0.5
06/16/80	4	13	ND	0.6
	5	4	ND	0.7
	6	9	ND	0.8
06/23/80	7	1	3	1.0
	8	1	5	1.0
	9	11	33	1.0
	10	1	7	1.0
	11	1	5	0.7

a = Diatomaceous earth

b = Used in the digestion process (n-Acetyl-L-Cysteine-NaOH
Method, Vestal, 1976)

c = Duplicate leachate digestion

d = Not determined

the Mycobacterium procedure suggested that the concentration procedure might also lead to C. perfringens isolation. Consequently, several methods were compared, namely: sulfite polymyxin sulfadiazine (SPS) agar (Angelotti et al., 1962), tryptone sulfite cycloserine (TSC) (Harmon et al., 1971), and iron milk stormy fermentation most-probable-number (MPN) test (Bonde, 1965; Dowell and Hawkins, 1976). The use of SPS agar was not satisfactory as the black colonies were not always apparent and there was no definite way to distinguish the presence of Clostridium perfringens from the contaminants as an agar layer covered the colonial growth. But on the tryptone sulfite cycloserine agar the C. perfringens colony types were apparent as they were large, 3 mm in diameter, mucoid, raised cream colored, and slightly opaque colonies which showed the presence of precipitated egg yolk surrounding the colony. These colonies were similar to those colonies on blood agar plates that had been inoculated with a culture of C. perfringens (ATCC #E3624). Five colonies from each sample were then identified. While the plates counts made from the TSC plates were used, they were not completely satisfactory as counts were sometimes low. Therefore, a second procedure was used in addition to the first. The procedure chosen was the stormy fermentation of iron milk (Bonde, 1965; Dowell and Hawkins, 1976). Furthermore, as several clostridia can produce stormy fermenta-

tion and many more can clot and digest milk, it was necessary to differentiate this bacterium from all the others. Therefore, 40% of the positive tubes were examined for the presence of C. perfringens. The identification procedures described in the following section were used. The latter procedure was used to separate C. perfringens from others as this bacterium grew at 45 C for five to six hours (Lennette et al., 1974), and would usually outgrow the others.

A comparison of the tryptone sulfite cycloserine (TSC) plates counts, incubated at 37 C for 24 hours, and the iron milk MPN levels, following incubation for 48 hours aerobically at 45 C and anaerobically within a GasPak (BBL) jar at 37 C for 48 hours, was made. The identification procedure selected was that of Washington (1981). All procedures have been fully described in Chapter 4. Table 46 shows that the Clostridium perfringens was present in most of the three tests. Following these results the TSC plates were chosen for continued use in this experiment along with the iron milk procedure incubated at 37 C in a GasPak jar as higher levels were obtained with this latter procedure than by incubation at 45 C.

TABLE 46. A COMPARISON OF ENUMERATION PROCEDURES TO DETERMINE THE PRESENCE OF CLOSTRIDIUM PERFRINGENS IN CONCENTRATED LYSIMETER LEACHATE

Samples	Clostridium perfringens Titters:		
	CFU/100 ml	MPN/100 ml	
	TSC plates ^a	Iron Milk Incubation at:	
	37 C at 24 hrs	45 C for 24 hrs Aerobically	37 C for 48 hrs Anaerobically ^b
Leachate: ^c			
UC-A	60	54	35
UC-B	< 1 X 10 ²	17	> 240
UC-C	< 1 X 10 ²	79	160
UC-E	70	11	54
Cultured			
<u>Clostridium perfringens</u> : ^d			
ATCC #E3624	1 X 10 ¹⁰	> 2.4 X 10 ⁹	9.2 X 10 ⁸

a = Tryptone sulfite cycloserine plates, incubated in a GasPak (BBL) jar for 24 hours and counted.

b = Incubated anaerobically in a GasPak (BBL) jar at 37 C.

c = UC-A, contained sewage sludge;

UC-B, contained a mixture of sewage sludge and municipal solid waste;

UC-C, contained municipal solid waste;

UC-E, contained a mixture of hospital waste and municipal solid waste.

d = Inoculum grown in cooked meat medium for 24 hours at 37 C in a GasPak (BBL) jar.

I. THE COMPARISON OF STREPTOCOCCAL ENUMERATION PROCEDURES

Fecal streptococcal levels had been determined routinely by the Standard Methods (1976) with the Gram stain for cocci, and frequently comparative levels were made using differential agar plates. At these times the plates usually gave higher titers. Consequently, a survey of eight tests, five of which were most-probable-number (MPN) tests and three were plate counts, were compared. The results are presented in Table 47, Parts I, II and III. As can be seen the low streptococcal levels in leachates ran about the same using all methods. These were from the Boone County lysimeters. On the other hand the Center Hill lysimeter leachates produced higher levels on the bile esculin agar than on the other agar plates. But, this same medium also gave non-specific results, i.e., colonies were present which contained Gram-positive rods and Gram-positive cocci. Therefore the bile-esculin agar was an unacceptable medium. The higher level leachate results can be seen on Table 47, part III. Note the average counts in the notes. Although this is not an acceptable means of examining these statistical counts the averaging will serve as a general means of comparison. These results show that the Standard Methods (1976) with the Gram stain for cocci and the bile esculin agar MPN results were the lowest. This is understandable to

TABLE 47. A COMPARISON OF LANDFILL (LYSINSTER) LEACHATE STREPTOCOCCAL LEVELS (PART I)

Leachate ^a Source	Procedure Number							
	1	2	3	4	5	6	7	8
	Streptococcal MPN per 100 ml			Direct Enumerative Plate Counts				
	Standard Methods (1976) No Stain Modified by Staining for Cocci	Recognized as Streptococci by Trans- fer of Azide Dextrose Growth to:			CPU per 100 ml			
			Bile Esculin Azide Agar (black) ^b	m-Enterococcus Agar (red) ^b R(red) ^b	KP Agar R(red) ^b	Bile Esculin Azide Agar	m-entero- cocci Agar	KP Agar
TC2A	< 2	< 2	130	130	20	< 10	< 10	ND ^c
TC2B	< 2	< 2	< 2	20	< 2	< 10	< 10	ND
TC2C	< 2	< 2	< 2	< 2	< 2	< 10	< 10	ND
TC2D	< 2	< 2	< 2	20	20	< 10	< 10	ND

a = TC2A, TC2B, TC2C, AND TC2D are from Walton, Kentucky. Samples taken on 10/23/79.

b = Colored colonial growth.

c = Not determined.

TABLE 47. A COMPARISON OF LANDFILL (LYSINETER) LEACHATE STREPTOCOCCAL LEVELS (PART II)

Leachate ^a Source	Procedure Number							
	1	2	3	4	5	7	8	
	Standard Methods (1976) No Stain		Streptococcal MPN per 100 ml		Direct Enumerative Plate Counts			
	Modified by Staining for Cocci	Recognized as Streptococci by Trans- fer of Azide Dextrose Growth to: Bile Esculin	m-Enterococcus Agar (Red) ^b	R (Red) ^b	CFU per 100 ml	CFU per 100 ml	KF Agar	
CH-1	< 2	< 2	23	< 2	< 2	< 10	< 10	
CH-2	< 2	< 2	< 2	< 2	< 2	< 10	< 10	
CH-3	23	< 2	240	< 2	< 2	50	< 10	
CH-4	< 2	< 2	4	7	20	< 10	< 10	
CH-5	< 2	< 2	< 2	< 2	< 2	< 10	< 10	
CH-6	< 2	< 2	50	< 2	< 2	< 10	< 10	
CH-7	< 2	< 2	< 2	< 2	< 2	< 10	< 10	
CH-9	< 2	< 2	< 2	< 2	< 2	100	< 10	
CH-11	< 2	< 2	9	< 2	< 2	< 10	< 10	
CH-12	< 2	< 2	< 2	< 2	< 2	50	< 10	
CH-13	< 2	< 2	< 2	< 2	ND ^c	< 10	< 10	
CH-14	49	20	< 2	< 2	ND	< 10	< 10	
CH-15	< 2	2	9	20	ND	< 10	< 10	
CH-16	2	< 2	6	2	ND	< 10	< 10	
CH-17	< 2	< 2	33	2	ND	< 10	< 10	
CH-18	< 2	< 2	330	< 2	ND	< 10	< 10	
CH-19	50	< 2	230	< 2	ND	< 10	< 10	
		< 2	2	< 2	ND	< 10	< 10	

^a = Center Hill, Cincinnati, Ohio. Samples taken on 10/16/79.

^b = Colored colonial growth.

^c = Not determined.

TABLE 47. A COMPARISON OF LANDFILL (LYSIMETER) LEACHATE STREPTOCOCCAL LEVELS (PART III)

Leachate ^a Sample Numbers	Procedure Number						
	1	2	3	4	5	6	7
	Streptococcal MPN per 100 ml			Direct Enumerative Plate Counts			
Standard Methods (1976) No Stain Modified by	Recognized as Streptococci by Trans- fer of Azide Dextrose Growth to:			CFU per 100 ml			
Staining for Cocci	Bile Esculin m-Enterococcus Agar (Red)b (Black)b			Bile Esculin m-antero- coccus Agar Agar			
CL1	9.4X10 ²	2.3X10 ²	5.0X10 ²	1.95X10 ³	1.8X10 ³	4.0X10 ³	1.1X10 ⁴
CL2	9.0X10 ²	9.0X10 ²	1.4X10 ³	1.9X10 ³	1.8X10 ³	3.4X10 ⁴	2.1X10 ⁴
CL3	7.5X10 ²	7.5X10 ²	3.5X10 ³	3.5X10 ⁴	1.7X10 ⁴	ND ^c	1.0X10 ⁴
CL4	9.2X10 ⁴	5.4X10 ⁴	3.5X10 ³	9.2X10 ⁴	9.2X10 ⁴	5.0X10 ⁴	7.4X10 ⁴
CL5	1.4X10 ³	4.9X10 ²	2.2X10 ⁴	1.6X10 ⁴	5.4X10 ³	ND	1.5X10 ⁴
CL6	9.4X10 ¹	6.0X10 ¹	ND	1.7X10 ³	1.7X10 ³	ND	3.0X10 ³
CL7	2.7X10 ³	2.7X10 ³	ND	2.4X10 ⁴	2.2X10 ³	ND	6.0X10 ⁴
CL8	5.4X10 ³	5.4X10 ³	ND	1.6X10 ⁴	1.6X10 ⁴	ND	1.8X10 ³

^a = As a means of comparison the eight methods average counts of this particular leachate series was calculated. These are: #1, 1.3X10⁴; #2, 8.0X10³; #3, 6.2X10³; #4, 2.5X10⁴; #5, 1.7X10⁴; #6, 2.9X10⁴; #7, 2.5X10⁴; and #8, 1.6X10⁴. Samples 1 through 8 were taken on 11/01/79 and the remainder on 04/14/80 from the Commercial Landfill sites CL-1 and CL-2.

^b = Colored colonial growth

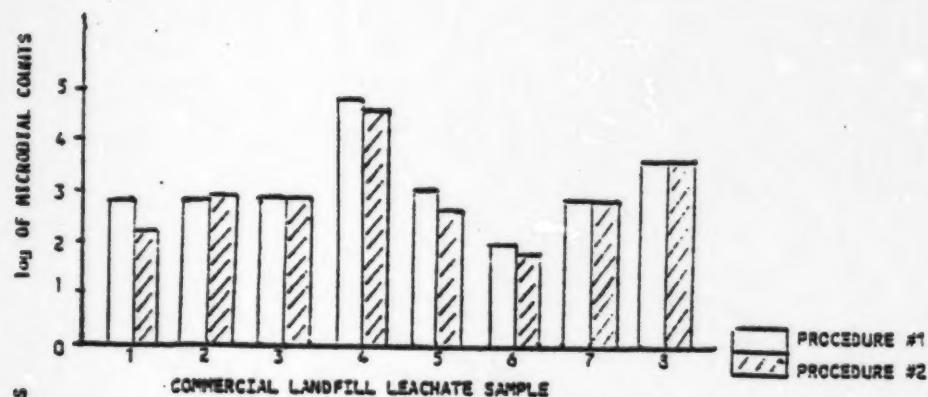
^c = Not determined

some degree as the Gram stain of the leachates remove all false-positive tests and is therefore more restrictive, which makes the counts lower. This decreased the total counts. Finally the m-enterococcus agar plate gave the highest plate count levels. Because of the high streptococcal levels this method was used on a limited basis to measure the fecal streptococci.

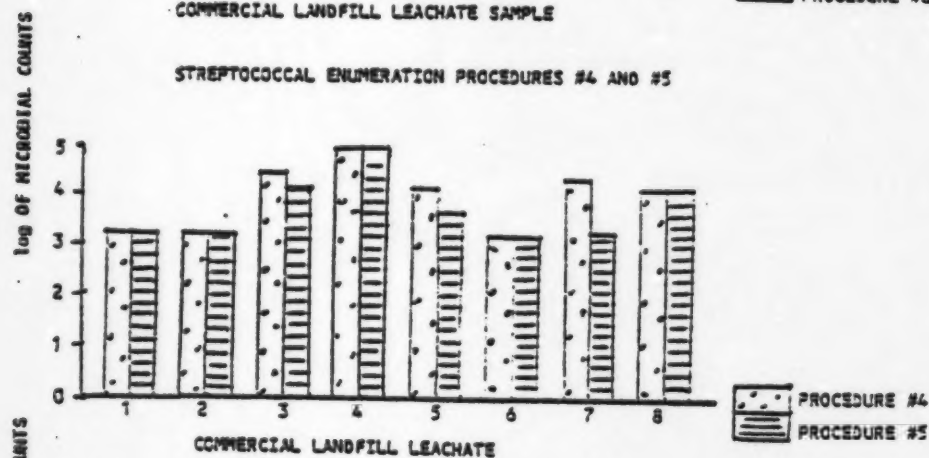
A further comparison of these eight methods is shown in Figure 20. The numbers on the left refer to the log of the plate counts while the bottom of the graphs indicate the leachate number. The procedural numbers are indicated by the markings on the bar graphs. Considering all three tables the results suggest that there is some variation between the various methods, especially between similar methods such as the MPN methods or the agar plate methods. The graphs at the top suggest that methods #1 and #2 gave lower levels than those using the differential agar plates. Finally, methods #7 and #8 demonstrated the higher results found using m-enterococcus agar. Usually they were used in conjunction with the Standard Methods (1976) with Gram stain procedure.

Finally, fresh leachate did bring about the stress of the fecal streptococci. This is shown in Table 48. Here five MPN methods are compared, i.e., methods #1 through #5.

STREPTOCOCCAL ENUMERATION PROCEDURE #1 AND #2



STREPTOCOCCAL ENUMERATION PROCEDURES #4 AND #5



STREPTOCOCCAL ENUMERATION PROCEDURES #7 AND #8

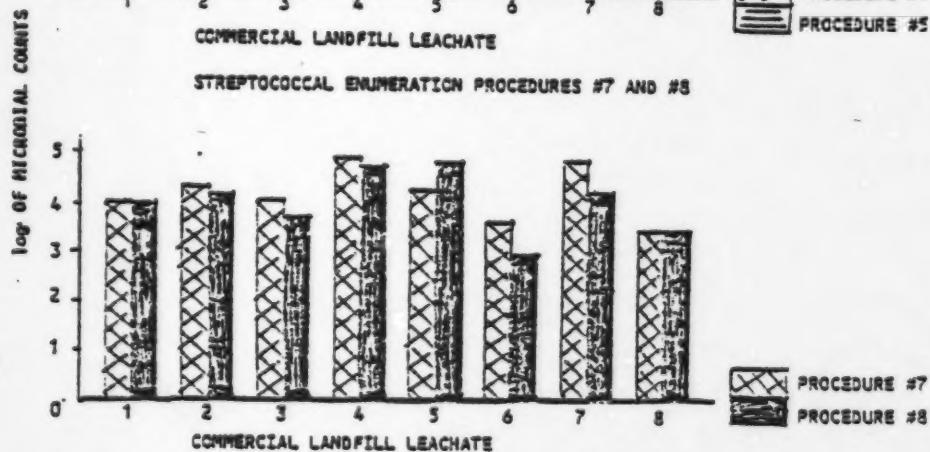


FIGURE 20. HISTOGRAM SHOWING THE COMPARISON OF STREPTOCOCCAL ENUMERATION PROCEDURES



TABLE 4B. POSITIVE FECAL STREPTOCOCCI MPN TEST RESULTS OF LANDFILL LEACHATES DISPLAYING BOTH A NORMAL AND AN INTERRUPTED ORDER OF POSITIVE TUBES WITH AN INCREASE IN DILUTION^a

Commercial Landfill Leachate Samples	Procedure Number				
	1	2	3	4	5
	Streptococcal MPN per 100 ml				
	Recognized as Streptococci by Transfer of Azide Dextrose Broth Growth to These Agars:				
Standard Methods (1976)	Modified by				
	No Stain	the Gram stain	Bile Esculin (black) ^b	m-Enterococcus (red) ^b	KF (red) ^b
I	<u>5-2-3-0^c</u>	5-0-0-0	<u>5-0-3-1</u>	<u>5-2-5-1</u>	5-3-3-1
II	<u>5-2-4-1</u>	<u>5-2-4-1</u>	5-3-2-0	<u>5-4-5-1</u>	<u>5-3-4-0</u>
III	<u>5-2-3-0</u>	<u>5-2-3-0</u>	5-5-1-0	5-5-5-1	5-5-4-1
IV	5-5-5-1	5-5-5-2	5-5-4-2	5-5-5-3	5-5-5-3

a = The 5-tube most probable number test (MPN) was used. The results displayed were the number of positive tubes per dilution (1.0, 10⁻¹, 10⁻² and 10⁻³, ml). The normal order of positive tubes per dilution is descending, as 5-4-3-1. An unusual (i.e., interrupted) order would be descending and then ascending, as 5-2-4-1.

b = Colored colonial growth.

c = The MPN tests showing the unusual order results are underlined. The number of leachate test results showing this order is: 9 of 20, or 45% of the total test results.

The bacterial stress in this case is demonstrated by the unusual number of positive results, i.e., they appear in reverse order. The higher concentrations of the leachate would be expected to produce the higher number of positive tubes with fewer numbers of positive results relating to the more dilute tubes. The reverse is true in this instance.

The number of MPN positive tubes given in Table 48 showed some irregularities. Normally, for a 5-tube test per dilution, and the use of four dilutions, the positive results, placed linearly starting with the highest dilution as 5-4-2-1, showed a decrease in positive results with an increase in dilution. An unusual order might be 5-2-4-1 indicating an inhibition of growth at the second dilution. The tests showing this unusual order of results are underlined. Nine out of the twenty results indicate this unusual condition. Thus, 45% of the tests had an unusual order of positive results. Such irregularities prevent the accurate translation of the number of positive results by the MPN statistical table to obtain the MPN/100 ml, because the normal distribution of bacteria with dilution has been interrupted. Of course, the results may be determined by the calculation method. Nevertheless, these results point to lower streptococcal levels in leachate by the MPN tests than are apparently present, and suggest the inhibition of growth during the testing process.

The MPN tests results are produced by bacterial growth that is inhibited to some degree. Therefore the streptococcal results are all much lower than they would be if some "toxic material" were not present. In other words, if the leachate escaped from the landfill and flowed into ground water where it was diluted, then the streptococcal levels would probably be much higher than the results shown in Table 48. A higher streptococcal count would indicate more extensive fecal pollution. Thus, the commercial landfill leachate will pollute the environment to a greater extent than the results indicate.

J. CONCLUSIONS

1. Without these improvements in the methods lower microbial levels and fewer microbial strains would have been isolated and identified.
2. The many improvements found indicated that many more could be added to these methods.
3. Water microbiology methods can never be substituted for solid waste methods without first adapting the procedures.
4. The Salmonella procedure should be further adapted and examined.

5. The best streptococcal method or methods should be determined and used in conjunction with the MPN test.
6. Stress methods should be considered for all assays.
7. A standard of methods should be established for solid waste assays.

K. SIGNIFICANCE OF THE RESULTS

The results show that these methods were studied only to a minimum degree, but the findings indicate that added effort on these procedures and close attention to details would greatly increase the pathogen and fecal indicator growth, isolation and identification. Also, the results suggest one cause of the problem is the stressed bacteria. These stressed bacteria are present in every sample. When allowed to repair they can grow as the nonstressed variety. However, such repair could happen away from the landfill when the leachate reached the ground water. The toxic inhibitors would be diluted out, increasing the pH and adding nutrients. The data suggest that the repair could increase the bacterial levels by a log or more.

In addition the MPN test was not designed for bacteria under stressed conditions or even leachate analysis. A revision of the procedures is greatly needed. The stress studies suggest that more fecal indicator

bacteria were present than this procedure indicates. Furthermore, the comparison of the MPN and plate counts show that the MPN test enumerates the lower levels while the plate counts count the higher levels. A combination of both procedures would present more accurate and precise streptococcal levels.

CHAPTER VI

RESULTS AND DISCUSSION: SIMULATED LANDFILLS

A. OVERVIEW

This section consists of results obtained, first, from existing units, and, secondly, from newly constructed units. First, the Boone County and the Center Hill simulated landfills (lysimeters), were assayed to determine the fecal indicator levels, plate counts, and the identification of pathogenic microorganisms. Secondly, the results from similar assays of solid waste and leachate were taken from the newly constructed lysimeters at the University of Cincinnati.

B. BOONE COUNTY AND CENTER HILL LYSIMETERS

1. Fecal Indicator and Plate Count Assays

Selected leachate samples from these lysimeters were examined periodically. The fecal indicator and plate counts assays are presented in Table 49. The ages of these lysimeters are noted on the table. The results show that the total coliform and the fecal coliforms had died out by this time. Notice that the eosin methylene blue plate counts included not only the Gram-negative rods but also a number of Gram-positive rods. In addition, the KF agar plate counts of 7.0×10^2 CFU/100 ml were higher than the MPN levels of fecal streptococci (80 MPN/100 ml). Finally, the

Table 49. MICROBIAL COUNTS^a FROM LANDFILL LEACHATE

Quantitative Tests	LANDFILLS (LYSIMETERS)		
	Boone County	Center Hill	
	TC-2,A-D (5 yrs) ^b	CH-19 (2 yrs) ^b	CH-9 (3 yrs) ^b
<u>MPN/100 ml</u>			
Total Coliforms	< 30	< 30	< 30
Fecal Coliforms	< 30	< 30	< 30
Fecal Streptococci	< 20	80	< 20
<u>Agar Plate Counts, CFU/100 ml</u>			
Blood-aerobic	5.0 X 10 ⁴	1.7 X 10 ⁵	5 X 10 ²
Blood-anaerobic	< 10 ³	1.8 X 10 ⁴	10 ³
Brain heart	1.0 X 10 ⁴	6.0 X 10 ⁵	10 ³
Eosin Methylene Blue	1.0 X 10 ⁴	9.0 X 10 ³	10 ²
Inhibitory Mold	7.0 X 10 ³	ND ^c	ND
KF Streptococcal	< 10 ³	7.0 X 10 ²	< 2
Mycosel ^d	10 ³	ND	10
Sabouraud ^d	2.0 X 10 ⁶	2.8 X 10 ⁴	6.0 X 10 ³
Tellurite	6.0 X 10 ³	7.0 X 10 ³	< 10

a = These are the averages of two or more leachate samples.

b = Age of waste in landfills and lysimeters.

c = Not determined.

d = Incubated at ambient temperature.

fungus levels were among the highest counts found, indicating perhaps aerobic conditions or contamination with aerobic material.

From these limited results it was difficult to determine the health significance of these lysimeters. So, a series of assays on lysimeter leachate were made over successive time periods. These results, presented in Table 50, are fecal streptococcal most-probable-assays number (MPN) assays, made over several years from the Center Hill lysimeters. They show that during a period of two to five years the numbers of lysimeters giving positive samples decreased from six to two lysimeters. This suggests that the streptococci were dying out. Also, previous to this the coliforms had disappeared. Thus, the disappearance of these fecal indicators suggested that perhaps the pathogens were also gone.

2. Microbial Identification

To give some measure of the pathogens that have survived, the microorganisms from these lysimeters were identified. The leachate bacteria isolated from these Boone County and Center Hill lysimeters are presented in Table 51. The results demonstrated that most of these isolates were saprophytes such as Penicillium. However, some pathogens were found.

TABLE 50. CENTER HILL LYSIMETER LEACHATE STREPTOCOCCAL CONCENTRATIONS

Lysimeter Designation	Concentration of Streptococci (MPN/100 ml)				Positive For Streptococci
	Age in Years				
	3	3	4	5	
CH-1	20	< 2X10 ³	40	< 2	+ ^a
CH-2	1100	< 2X10 ³	37	< 2	+
CH-3	< 2	< 2X10 ³	< 2	< 2	- ^b
CH-4	< 2	< 2X10 ³	< 2	< 2	-
CH-5	< 2	< 2X10 ³	< 2	< 2	-
CH-6	< 2	< 2X10 ³	< 2	< 2	-
CH-7	< 2	2X10 ³	< 2	< 2	+
CH-8	< 2	< 2X10 ³	11	ND ^c	+
CH-9	< 2	< 2X10 ³	ND	< 2	-
CH-10	< 2	< 2X10 ³	< 2	ND	-
CH-11	< 2	2X10 ³	< 2	< 2	+
CH-12	1300	3.3X10 ⁴	< 2	< 2	+
CH-13	< 2	< 2X10 ³	< 2	< 2	-
CH-14	800	4X10 ³	< 2	20	+
CH-15	< 2	< 2X10 ³	ND	< 2	-
CH-16	< 2	< 2X10 ³	< 2	2	+
CH-17	< 2	< 2X10 ³	< 2	< 2	-
CH-18	230	< 2X10 ³	< 2	< 2	-
CH-19	230	1.3X10 ⁴	ND	< 2	+
No. of Positive Lysimeters	6	5	3	2	9

a = Positive

b = Negative

c = Not determined

TABLE 51. MICROORGANISMS ISOLATED FROM BOONE COUNTY AND
CENTER HILL LEACHATE

Site	Leachate Source	Microbial Isolates Identification ^a	Number of Strains
Boone County	TC2 (A-D)	<u>Allescheria boydii</u>	
		<u>Bacillus</u> sp.	
		CDC Ve-1	
		<u>Cephalosporium</u> sp.	
		<u>Corynebacterium</u> sp.	
		<u>Listeria monocytogenes</u>	
		<u>Micrococcus</u> sp.	
		<u>Moraxella</u> sp.	
		<u>Streptococcus durans</u>	2
		<u>Streptococcus</u> , Type Q	8
Center Hill	CH-9	<u>Streptococcus faecalis</u>	12
		<u>Bacillus</u> sp.	
		<u>Corynebacterium</u> sp.	
		<u>Listeria monocytogenes</u>	
		<u>Sepedonium</u> sp.	
		<u>Streptococcus</u> sp.	
		Yeast	
	CH-19	<u>Acinetobacter</u> sp.	
		<u>Alcaligenes</u> sp.	
		<u>Bacillus</u> sp.	
		<u>Clostridium</u> sp.	
		<u>Escherichia cloacae</u>	
		<u>Fusarium</u> sp.	
		<u>Penicillium</u> sp.	
		<u>Pseudomonas</u> -like	
		<u>Staphylococcus</u> sp.	
		<u>Streptococcus</u> sp.	
		Yeasts	
	CH-14	<u>Enterococcus</u> , Type D	8
		Lactic <u>Streptococcus</u>	2

a = Methods of microbial identification were: Gram-positive rods, Cowan (1974), Gram-negative rods, Wolf et al. (1973); Streptococcus, Pavlova et al. (1972); and Fungi, Koneman et al. (1978) with one exception E. cloacae was identified by API-20E.

Listeria monocytogenes was present which produces listeriosis and which may produce meningitis, septicemia and other manifestations. Acinetobacter sp. is also an opportunistic pathogen which produces meningitis and septicemia. Moraxella sp. is an opportunistic pathogen which is frequently involved in severe infections. Allescheria boydii is one of several species which produces a condition called madura foot.

These opportunistic pathogens are not considered to be highly infectious, but they can be very pathogenic. These pathogens easily infect individuals who are aged, sick with chronic diseases such as fungal diseases or cancer, have immunological disorders, and are undernourished. Most healthy people are not so affected by these microorganisms. Finally, twelve strains of Streptococcus faecalis were isolated and were differentiated from S. faecalis var. liquefaciens by biochemical means. The latter are usually found on vegetation and thus have no health significance. On the other hand the S. faecalis does have health significance in that they are derived from humans and animals. Their isolation would indicate the presence of other human and animal microorganisms.

C. UNIVERSITY OF CINCINNATI LYSIMETERS

1. Analysis of Solid Waste Used in Lysimeter Construction

The microbial analysis of the three types of solid wastes used in the preparation of the six lysimeters are presented in table 52. These results show that all three wastes had high levels of total coliforms, fecal coliforms, and fecal streptococci. In general the sewage sludge gave lower fungal levels than the others. This would be expected because the microbial population was restricted to those coming from the digestive tract. The municipal solid waste on the other hand contained high fungal counts probably because it contained waste from a wider source such as soil, trash, or sweepings.

To further identify the above waste the fecal coliform/fecal streptococci (FC/FS) ratio was used to define the human or animal fecal origin of the wastes (Geldreich et al., 1969; and Feachem, 1975). Geldreich et al. had determined that environmental samples from human fecal material gave FC/FS ratios of >4 while animals gave ratios of less than 0.7.

TABLE 52. LANDFILL SOLID WASTE MICROBIAL ANALYSES YIELDING
FECAL INDICATOR LEVELS AND AGAR PLATE COUNTS

Parameters	The Number of Microorganisms in Waste Added to Lysimeters:		
	Sewage Sludge	Hospital Waste	Municipal Waste
Fecal Indicator Tests ^a (MPN/100g)			
Total Coliforms	2.8X10 ¹¹	9.0X10 ¹⁰	7.7X10 ¹⁰
Fecal Coliforms	2.4X10 ¹⁰	9.0X10 ¹⁰	4.7X10 ¹⁰
Fecal Streptococcus	3.3X10 ⁹	8.6X10 ¹⁰	2.5X10 ¹¹
Agar Plate Counts (CFU/100g)			
Standard Methods	1.7X10 ¹⁰	3.8X10 ¹⁰	4.3X10 ¹¹
Blood - Aerobic	4.1X10 ¹⁰	3.9X10 ¹⁰	3.6X10 ¹¹
Blood - Anaerobic	2.9X10 ¹⁰	2.2X10 ¹⁰	3.5X10 ¹¹
Eosin Methylene Blue	1.5X10 ¹⁰	3.1X10 ¹⁰	3.4X10 ¹⁰
Inhibitory Mold Agar	1.0X10 ⁷	3.8X10 ⁸	6.9X10 ⁹
KF Streptococcal	3.6X10 ⁷	3.0X10 ⁹	4.2X10 ¹⁰
Mycosel	3.6X10 ⁷	7.5X10 ⁹	1.62X10 ⁹
Sabouraud	1.4X10 ¹⁰	3.4X10 ¹⁰	2.5X10 ¹¹
Tellurite	4.3X10 ⁷	2.6X10 ⁸	6.6X10 ⁹

a = Standard Methods (1976) MPN tests used for the coliforms and for the fecal streptococci. The positive tests were examined for Gram-positive cocci.

The results of applying the FC/FS ratio to the sewage sludge, municipal solid waste and hospital waste fecal indicator levels are shown in Table 53. The ratios according to Geldreich et al (1969) and Faechem (1975) indicated that only the sewage sludge had a human origin while the municipal solid waste was of animal origin or at least of nonhuman origin (Van Donsel and Geldreich 1971). The hospital waste with the ratio of 0.11 indicated the nonhuman or animal origin of the waste. One explanation for these results was that the hospital waste was dry, due to the amount of paper products that were present in the waste. Perhaps this could have decreased the fecal coliform levels (prior to its inclusion in the lysimeters), which in turn resulted in a decrease in the FC/FS ratio. In addition the municipal waste was expected to be high in fecal material because of the pet feces and baby's diapers, but the ratio was low, suggesting an animal origin. However, the long municipal waste collection period in August with its high temperatures in Columbus, Ohio, the shredding, and the trip to Cincinnati could also have decreased the ratio.

The microorganisms isolated and identified from each waste type is presented in Table 54. Six of the fifteen identified genera are listed as pathogens. Most of these bacteria are usually found in feces (Scherago, 1972; Davis

TABLE 53. COMPARISON OF FECAL COLIFORM AND FECAL STREPTOCOCCI TITERS TO DETERMINE THE ORIGIN OF FECAL POLLUTION OF SOLID WASTE IN UNIVERSITY OF CINCINNATI LYSIMETERS

Type of Solid Waste	Fecal Coliform/ Fecal Streptococci Ratios ^a
Sewage Sludge	$\frac{2.4 \times 10^{10}}{3.3 \times 10^9} = 7.27$
Hospital Waste	$\frac{9.0 \times 10^{10}}{8.6 \times 10^{10}} = 1.05$
Municipal Waste	$\frac{4.7 \times 10^{10}}{2.5 \times 10^{11}} = 0.19$

a = Ratios of Most-Probable-Number (MPN) values as described by Geldreich et al., (1964).

TABLE 54. GRAM-NEGATIVE BACTERIA ISOLATED FROM SOLID WASTE USED IN THE CONSTRUCTION OF THE UNIVERSITY OF CINCINNATI LYSIMETERS

Bacterial ^a Genera and Species	Bacterial Numbers Isolated from		
	Sewage Sludge	Municipal Waste	Hospital Waste
Oxidase Negative			
Lactose Fermenters	<u>6</u>	<u>10</u>	<u>7</u>
<u>Escherichia coli.</u>	8	3	2
<u>Enterobacter</u> sp.	7	3	6
<u>Klebsiella</u> ^b sp.	3	3	5
<u>Citrobacter</u> sp.	<u>4</u>	<u>10</u>	<u>11</u>
Sub-total	22	19	24
Oxidase Negative			
Non-lactose Fermenters			
<u>Serratia</u> sp.	4	c	4
<u>Proteus</u> sp.	3	10	13
<u>Providencia</u> sp.	c	1	1
<u>Salmonella</u> ^b sp.	<u>c</u>	<u>3</u>	<u>1</u>
Sub-total	7	14	19
Oxidase Positive			
Nonfermenters			
<u>Aeromonas</u> ^d sp.	c	2	3
<u>Flavobacterium</u> sp.	1	c	c
<u>Herellea</u> ^b sp.	c	2	2
<u>Mima</u> ^b sp.	2	1	c
<u>Moraxella</u> ^b sp.	c	1	1
<u>Pasteurella hemolyticum</u> ^b	1	c	c
<u>Pseudomonas</u> sp.	<u>1</u>	<u>2</u>	<u>17</u>
Sub-total	5	8	23
Total	34	41	66

a = Identified by the procedure of Wolfe (1975).

b = These bacteria appear on the Communicable Disease Center (1974) list, i.e., Classification of Etiologic Agents on the Basis of Hazard.

c = None detected.

d = A fermenter.

et al. 1980; and Wilson & Miles, 1975). Of the six pathogen types listed, three were found in sewage sludge, five in municipal waste, and four in hospital waste. Of the total number of pathogens isolated from each waste, six were from sewage sludge, ten were from municipal waste, and eight were from hospital waste.

In conclusion, these three types of wastes had a similar microbial composition. However, the FC/FS ratio varied due to the presence of large amounts of fecal material in the sewage sludge, and none (or less) in the others.

2. Analysis of Leachate from the Lysimeters

a. Fecal Indicator Levels

The results of the fecal indicator tests taken over a two year span are presented in Table 55 and 56. They show that most total coliforms and fecal coliforms MPN levels had decreased to < 20 MPN/100 ml at the end of the seventh week and the fecal streptococci had disappeared by the end of the first year. After the fecal indicator levels had disappeared, a procedural change (Allen et al., 1976) was made at the 13th week which increased the levels of four out of six lysimeters, i.e., in all but UC-E and UC-F. While these values were low they did indicate that the fecal indicators had not died as the results in Tables 55 and 56

TABLE 55. COLIFORM LEVELS IN LEACHATES FROM THE UNIVERSITY OF CINCINNATI
LYSIMETERS^a

Total Coliform ^c (& Fecal Coliform) MPN/100 ml of Lysimeter Leachate						
Week ^b	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
4	5 (2)	1.4X10 ⁵ (9X10 ⁴)	1.3X10 ³ (7.9X10 ²)	1.3X10 ⁴ (1.4X10 ³)	— ^d —	— —
5	< 20 (< 20)	1.3X10 ² (5X10 ¹)	1.2X10 ² (2X10 ¹)	2 (< 2)	1.3X10 ³ (1.3X10 ²)	2.4X10 ³ (2.4X10 ³)
7	< 20 (< 20)	7.0X10 ² (2.0X10 ²)	< 20 (< 20)	< 20 (< 20)	< 20 (< 20)	< 20 (< 20)
9	< 20 (< 20)	< 20 (< 20)	< 20 (< 20)	< 20 (< 20)	< 20 (< 20)	< 20 (< 20)
11	< 20 (< 20)	< 20 (< 20)	< 20 (< 20)	< 20 (< 20)	< 20 (< 20)	< 20 (< 20)
13	20 (< 20)	82 (< 20)	40 (< 20)	< 20 (< 20)	< 20 (< 20)	< 20 (< 20)
15	220 70	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)
17	130 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)
19	170 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)
22	50 (< 2)	2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)
27	11 (< 2)	2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)
31	11 (2)	< 1 (< 1)	< 1 (< 1)	< 1 (< 1)	< 1 (< 1)	< 1 (< 1)
40	1 (< 2)	4 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)
54 & 69	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)
100	49 (< 2)	2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)	< 2 (< 2)

a = Lysimeter contents: UC-A = Sewage Sludge; UC-B = Municipal Solid Waste plus Sewage Sludge; UC-C & UC-D = Municipal Solid Waste; UC-E = Hospital Waste plus Municipal Solid Waste; UC-F = Hospital Waste.

b = Weeks after lysimeter construction.

d = Not determined.

c = As indicated in Standard Methods (1976). The Allen et al., (1976) technique was used on the 13th week and thereafter.

TABLE 36. STREPTOCOCCAL MPN TITERS IN LEACHATES FROM THE UNIVERSITY OF CINCINNATI LYSIMETERS^a

Streptococci MPN ^c /100ml of Leachate from Lysimeters						
Week ^b	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
4	7.9X10 ²	4.9X10 ⁵	1.7X10 ⁵	3.3X10 ⁶	— ^d	—
5	9.2X10 ³	9.2X10 ²	5.4X10 ⁷	2.4X10 ⁷	5.4X10 ⁶	5.4X10 ⁷
7	7.9X10 ²	9.2X10 ⁴	2.2X10 ⁶	3.2X10 ⁵	1.7X10 ⁵	1.3X10 ⁶
9	2.4X10 ³	1.6X10 ⁴	5.4X10 ⁷	2.0X10 ³	1.4X10 ⁵	5.0X10 ⁴
11	3.5X10 ³	3.4X10 ²	< 2X10 ⁴	< 2.0X10 ³	1.7X10 ³	< 2.0X10 ³
13	4.9X10 ²	7.9X10 ²	3.5X10 ⁵	2.0X10 ¹	2.6X10 ⁴	1.3X10 ²
15	3.3X10 ²	2.3X10 ³	8X10 ²	< 20	1.1X10 ²	2.2X10 ²
17	1.7X10 ²	1.1X10 ³	2.4X10 ⁴	3.4X10 ³	2.4X10 ⁴	2.2X10 ²
19	50	700	1,100	1,400	2.9X10 ⁵	130
22	< 2	790	7.0X10 ³	< 20	2.0X10 ³	5.0X10 ²
27	80	9.2X10 ⁴	> 1.6X10 ⁵	4.9X10 ³	1.7X10 ⁴	50
31	5.4X10 ²	2.4X10 ³	1.1X10 ³	50	2.2X10 ²	< 2
35	< 20	1.2X10 ²	3.3X10 ²	2.0X10 ¹	1.1X10 ²	< 20
40	< 2	9	1.3X10 ²	2.0X10 ¹	7.9X10 ²	< 2
54	< 2	20	1.4X10 ²	< 2	1,400	< 2
69	< 2	2	< 2	< 2	< 2	< 2
100	< 2	2	< 2	< 2	< 2	< 2

a = Lysimeter contents: UC-A = Sewage Sludge; UC-B = Municipal Solid Waste plus Sewage Sludge; UC-C & UC-D = Municipal Solid Waste; UC-E = Hospital Waste plus Municipal Solid Waste; UC-F = Hospital Waste.

b = Weeks after lysimeter construction.

c = Standard Methods (1976) plus microscopic examination of positive tubes Gram-positive cocci.

d = Not determined.

had indicated previously. However, the meaning of these low levels were not clear at this time. The presence of low levels of the total coliforms did continue through the 100th week from lysimeter UC-A (containing sewage sludge) and lysimeter B (containing sewage sludge and municipal solid waste).

A chance observation at lysimeter age of 2-1/2 years showed that the fecal indicator bacteria had not completely died out. The fecal indicator levels at that time were low or apparently nonexistent, yet fecal indicator survival was found when one of the six lysimeters (UC-A containing sewage sludge) was examined daily after it first gave lower weekly volumes of leachate (see Table A-1 in the Appendix) and then several weeks later gave higher than usual volumes of leachate. We speculated that the tubing by which the leachate drained from the lysimeter to the outside was plugged with a small portion of sewage sludge, and which later became unplugged, allowing the remaining leachate to drain out. Then this same sequence of events happened again several weeks later. This second time we ran indicator levels daily on the leachate when the leachate began to flow in regular volumes again. These results are presented in Table 57. As before the leachate was drawn off daily instead of weekly to see if higher fecal indicator levels would be present in the leachate following the dislodgement

TABLE 57. UNUSUALLY HIGH LEACHATE FECAL INDICATOR CONCENTRATIONS FROM THE SEWAGE SLUDGE LYSIMETER (UC-A) TWO YEARS OLD.

Lysimeter ^a Age (week-day)	Fecal Indicators ^b (MPN/100 ml)		
	Total Coliforms	Fecal Coliforms	Fecal Streptococci
98	> 2,400	< 2	> 2,400
102-0	1,400	< 2	424
102-2	9,200	< 2	17,400
102-3	> 24,000	< 2	29,800
102-4	> 24,000	< 2	16,600
102-5	> 24,000	< 2	8,400
102-6	35,000	< 2	39,000
113-0	700	< 2	1,700
113-1	557	5	16,000
113-2	5	5	< 2
113-7	2,800	49	5,400
115	1,400	< 2	16,000
116-0	9,200	< 2	17,400
116-1	> 24,000	< 2	29,000

a = Lysimeter was build on 8/16/78.

b = The total coliform and the fecal coliform MPN tests were those described by Standard Methods (1976) modified by the Allen et al., (1976) procedure, and the fecal streptococci MPN procedure was modified by examining all positive ethyl violet azide tubes for Gram-positive cocci.

of portions of the sewage sludge revealing a new sludge surface with higher fecal indicator levels. The results did show higher MPN fecal indicator levels and perhaps the above speculation was true. These MPN levels were as high as those seen when the lysimeters were four to five weeks old. An alternative view was that the fecal indicator levels were always present but they died during their weekly stay in the bottom of the lysimeter. If the latter view is true then the weekly water addition washed the fecal indicator bacteria from the sewage sludge into the leachate. They were then measured by the daily MPN tests instead of weekly. Thus, when the routine weekly assays were done the fecal indicator bacteria were not alive, but had been killed by acid or other toxic agents. The conclusion made earlier that all fecal indicators had died out within the first year was in light of these conclusions probably incorrect. Furthermore, if these recent conclusions were correct and if the fecal indicator bacteria were truly alive after two to three years in lysimeters and drained downward with the leachate, then they could be a pollution source to ground water over a longer period of time than previously thought. Finally, these results are particularly important, as no additional contamination could have occurred as those in the field from wild animals defecating on the surface of the landfill and storm water washing the fecal material into the

waste, because this waste was contained in sealed 55-gallon drums and kept in a laboratory.

b. Microbial Plate Counts

As the fecal indicator bacteria in the lysimeters decreased with time so did the microorganisms growing on the differential agar plates as shown in Table 58. In comparing the aerobic and anaerobic blood agar plate the anaerobic blood agar plate counts did not show (at the 100th week) an increase in microbial levels as expected due to the presence of anaerobic conditions. Instead the anaerobic plate levels were unusually low indicating a predominance of anaerobes. The purpose of these latter studies was to grow Clostridium perfringens , but none were found by this procedure. Also, the plate counts from the Standard Methods agar demonstrated that high levels of microorganisms were present. Furthermore, the Sabouraud agar counts showed that the fungi levels decreased by one to two logs between the 13th and the 100th week. These fungi were aerobic. In some way perhaps they were inhibited from growing during the later weeks. Similarly, the nonsporeforming Gram-positive rods also decreased, this time by two to three logs. Finally, considering the table as a whole, most of the bacterial types found in the lysimeter died to some degree during the experiment, except for those from lysimeter A. These sewage sludge microbes not only survived, but increased in numbers

TABLE 59. MICROBIAL CONCENTRATIONS IN LEACHATES OBTAINED FROM THE LABORATORY LYSIMETERS AT THE 13th AND THE 100th WEEK AFTER CONSTRUCTION

Quantitative Tests	UC Laboratory Lysimeters ^a						
	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F	UC-P
Fecal Indicator Tests, MPN/100 ml							
Total Coliform ^b	20 (49) ^c	82 (2)	40 (< 2)	< 20 (< 2)	< 20 (< 2)	< 20 (< 2)	< 20 (< 2)
Fecal Coliform ^b	< 20 (< 2)	< 20 (< 2)	< 20 (< 2)	< 20 (< 2)	< 20 (< 2)	< 20 (< 2)	< 20 (< 2)
Fecal Streptococci ^d	4.9x10 ² (< 2)	7.9x10 ² (2)	3.5x10 ² (< 2)	2.0x10 ¹ (< 2)	2.6x10 ⁴ (< 2)	1.3x10 ² (< 2)	1.3x10 ² (< 2)
Agar Plates, CFU/100 ml							
Blood - aerobic	1.9x10 ⁷ (1.2x10 ⁸)	1.2x10 ⁸ (1.3x10 ⁶)	6.8x10 ⁷ (1.6x10 ⁶)	2.7x10 ⁸ (1.4x10 ⁷)	7.6x10 ⁶ (9.5x10 ⁵)	1.2x10 ⁷ (3.3x10 ⁵)	1.2x10 ⁷ (3.3x10 ⁵)
Blood - anaerobic	6.3x10 ⁵ (1.2x10 ⁶)	1.1x10 ⁷ (3.3x10 ⁶)	8.0x10 ⁷ (3.2x10 ⁶)	5.6x10 ⁷ (4.0x10 ⁶)	3.1x10 ⁷ (1.7x10 ⁶)	2.1x10 ⁸ (2.7x10 ⁶)	2.1x10 ⁸ (2.7x10 ⁶)
Standard Methods	1.4x10 ⁸ (1.0x10 ⁹)	5.4x10 ⁷ (1.4x10 ⁶)	7.9x10 ⁷ (1.2x10 ⁸)	3.1x10 ⁸ (2.2x10 ⁷)	1.5x10 ⁷ (2.8x10 ⁷)	6.3x10 ⁸ (3.1x10 ⁴)	6.3x10 ⁸ (3.1x10 ⁴)
Sabouraud	2.2x10 ⁷ (3.1x10 ⁶)	4.9x10 ⁷ (4.2x10 ⁵)	2.2x10 ⁷ (4.2x10 ⁵)	6.6x10 ⁷ (1.7x10 ⁶)	4.6x10 ⁷ (3.5x10 ⁵)	5.2x10 ⁶ (1.9x10 ⁴)	5.2x10 ⁶ (1.9x10 ⁴)
Tellurite	1.1x10 ⁶ (4.0x10 ³)	1.6x10 ⁶ (2.9x10 ⁴)	7.8x10 ⁶ (4.0x10 ³)	6.0x10 ⁵ (1.6x10 ⁴)	1.7x10 ⁶ (6.4x10 ⁴)	5.9x10 ⁶ (< 10 ³)	5.9x10 ⁶ (< 10 ³)

a = University of Cincinnati (UC) lysimeter contents; UC-A, sewage sludge; UC-B, municipal solid waste plus sewage sludge; UC-C, municipal solid waste; UC-D, municipal solid waste; UC-E, municipal solid waste plus hospital waste; and UC-F, hospital waste.

b = Standard Methods (1976), and at the 13th week modified by Allen et al (1976)

c = The microbial counts after 100 weeks are in the parentheses.

d = Standard Methods (1976) plus microscopic examination of positive tubes for Gram-positive cocci.

as demonstrated by the counts made on the blood agar plates. Perhaps there was some bacterial multiplication. It is known that bacteria grow nicely at 20 C (Stanier et al., 1976). More data is presented in Appendices E and F.

The lack of anaerobe increase on blood plates remained a mystery. Consequently, assays of lysimeter gas were made to determine the composition. This is shown in Table 59. A comparison of gases from October 1978 and a date six months later indicates that the lysimeters definitely became more anaerobic with time and even produced methane, although lysimeter F still had great quantities of oxygen, indicating that few aerobes were present to utilize the oxygen.

Finally, the leachate was concentrated to detect the presence of Clostridium perfringens. The results are presented in Table 60. The TSC (tryptone sulfite cycloserine) plates gave only low levels of C. perfringens while the MPN tubes gave higher levels. These MPN concentrations were approximately ten times higher than those shown on the TSC plates. These low levels of C. perfringens enumerated by plate counts on differential agar demonstrate why difficulties were present in isolating these microorganisms on blood agar plates. Perhaps the high concentration of bacteria including the saprophytes present in the leachate may have interfered with the colonial formation or the

TABLE 59. ANALYSIS OF LABORATORY LYSIMETER GAS SAMPLES

Lysimeter ^a Designation	Lysimeter Age in Weeks	Date of Sample	Percentage of Gas ^b Present			
			CO ₂	O ₂	N ₂	CH ₄
UC-A	8	10/27/78	12	14	72	1
UC-B			18	13	64	3
UC-C			33	12	54	0
UC-D			55	6	38	0
UC-E			55	6	37	0
UC-F			5	18	75	0
UC-A	32	04/04/79	58	0.03	34	6
UC-B			62	1.0	22	13
UC-C			72	1.0	19	6
UC-D			67	0.78	17	14
UC-E			64	0.87	12	21
UC-F			11	12.0	75	0.5
UC-E	64	11/26/79	67	1.0	17	13

a = The contents of lysimeters are: UC-A, sewage sludge; UC-B, municipal solid waste plus sewage sludge; UC-C, municipal solid waste; UC-D, municipal solid waste; UC-E, municipal solid waste plus hospital waste; and UC-F, hospital waste.

b = Analyzed with a gas chromatograph.

TABLE 60. A PRELIMINARY DETERMINATION OF CLOSTRIDIUM PERFRINGENS LEVELS AND OTHER PARAMETERS FROM FOUR
UNIVERSITY OF CINCINNATI LYSIMETERS^a LEACHATE

Sample Sources	Clostridia perfringens level in concentrated leachate		Leachate	pH	Conductivity
	TSC ^b plates, CFU/100 ml	Iron Milk, MW/100 ml	Volume(L)		(umhos/cm)
Leachate ^c					
UC-A	6	35	2.1	6.7	2619
UC-B	< 1 x 10 ²	>240	2.13	6.6	1917
UC-C	< 1 x 10 ²	160	2.12	4.8	1789
UC-E	6	54	2.11	5.5	1220
Cultured Clostridium perfringens ATCC #3624	1 x 10 ⁸	9.2 x 10 ⁸	NA ^e	NA	NA

a = The lysimeters were 2-3/4 years old.

b = Tryptone sulfite cycloserine agar.

c = Concentrated by diatomaceous earth filtration.

d = Grown in cooked meat medium in a GasPak (BBL) jar for 24 hours.

e = Not applicable.

double zone hemolysis for Clostridium recognition on blood agar plates (anaerobic). Moreover, these microorganisms had to be concentrated in the leachate before they could be found. The leachate was concentrated 100 times. The concentration was brought about by filtering 2.1 L of leachate through a diatomaceous earth filter. Also of concern was the pH. The pH varied from 6.7 to 4.8, which is important as the fecal bacteria could probably survive in a pH of 6.7, but probably not at pH 4.8. The higher pH shown by UC-A and UC-B suggests that sewage sludge may have had more of a buffering capacity than the other wastes as their pH was lower.

c. Microbial Identification

Streptococcal species were identified to determine not only their survival, denoting the presence of fecal material, but also to indicate the origin of the feces. In the analysis of streptococcal species, Table 61 compares these bacteria from solid waste placed in the University of Cincinnati lysimeter with those isolated from the leachate during the fourth through ninth months after construction. Thus, the solid waste had a greater variety of streptococci than the leachate. This would indicate that not all streptococci survive in the lysimeters or survive to the same extent. In fact, the municipal waste had a greater variety of streptococci than the hospital waste. This seems

TABLE 61. STREPTOCOCCAL GROUPS^a AND SPECIES^a ISOLATED FROM SOLID WASTE USED IN THE LYSIMETERS^b AND FROM THE LEACHATE AFTER CONSTRUCTION^c

Streptococcal Group	Number of Streptococcal Isolates From:								
	Solid Waste		Lysimeter ^b Leachate						Total
	Municipal	Hospital	A	B	C	D	E	F	
Group A	ND ^d	ND	ND	ND	ND	ND	ND	ND	ND
Group B	1	ND	ND	ND	ND	ND	ND	ND	1
Not Groups A, B, or D (i.e., Group C, G and/or F)	2	1	ND	ND	ND	ND	ND	ND	3
Group D (enterococcus)									
<u>S. faecalis</u> var. <u>liquefaciens</u>	3	5	ND	ND	ND	ND	9	10	27
<u>S. faecalis</u>	1	ND	1	1	ND	ND	3	8	14
<u>S. faecium</u>	1	ND	4	9	4	ND	7	ND	25
<u>S. durans</u>	ND	ND	ND	7	3	ND	1	4	15
TOTAL Group D	5	5	5	17	7	ND	20	22	81
Group D (Not enterococcus)	1	2	2	ND	5	6	4	1	21
Viridans Group	3	4	6	2	ND	ND	1	1	17
<u>S. salivarius</u>	2	1	ND	ND	ND	ND	ND	ND	3
TOTAL Viridans Group	5	5	6	2	ND	ND	1	1	20

a = The streptococcal groups were identified by the procedure of Facklam, et al. (1974), and the species were identified by the procedure of Pavlova, et al. (1972).

b = The contents of lysimeters are: UC-A, sewage sludge; UC-B, municipal solid waste plus sewage sludge; UC-C, municipal solid waste; UC-D, municipal solid waste; UC-E municipal solid waste plus hospital waste; UC-F, hospital waste.

c = Taken during weeks nine through twenty following lysimeter construction.

d = None detected.

reasonable as municipal solid waste must have a far greater variety of material present in the waste than hospital waste. In a comparison of Table 61 with that on the origin of the streptococci, Table 26, the results indicated that municipal solid waste contained streptococci identified (see Tables 61 and 62) as Group B, and C, G, or F along with enterococcus Group D and the nonenterococcus Group D, indicating that these strains came from animals. These cocci could have come from pet feces or from animals used for food. The S. faecalis var. liquefaciens originated from vegetables and connotes no health hazard. On the other hand, the viridans group is derived from man and indicated a possible health hazard. A comparison of this Table 26 with Table 61 indicates that the Groups C, F, and G streptococci are of animal origin, and that they did not survive the lysimeter conditions. The rest of the streptococci are found in human or animal feces. The Group D nonenterococcus species are found in horses and cows. The viridans groups are found in the human mouth and milk. In conclusion, all group D streptococci survived in large numbers indicating that along with the presence of these human indicator bacteria that other human microorganisms of similar resistance will also be found in the leachate, and finally human fecal material is present in these wastes.

TABLE 62. THE PRESUMPTIVE IDENTIFICATION OF STREPTOCOCCAL GROUPS^a AND SPECIES^b ISOLATED FROM LANDFILL LEACHATES

Grouping Parameters ^a					Isolates:	
Strepto- coccal Sources	Sensi- tivity to Bacitracin	Hip- purate Test	Growth on:		Number	Identification ^b
			Bile Esculin	6.5% NaCl		
Lysimeter Leachates:						
UC-E	- ^c	-	+ ^d	+	14	D-enterococcus (6 <u>S. faecalis</u>)
UC-B	-	-	+	+	2	D-enterococcus (2 <u>S. faecalis</u>)
<u>Streptococcus</u> <u>faecalis</u>	-	-	+	+	1	D-enterococcus
ATCC 19433					Total 17	

a = Facklam et al. (1974)

b = Pavlova et al. (1972), Standard Methods (1976), Bordner et al. (1978)
for the species determinations

c = Negative

d = Positive

The identification of the microorganisms from the lysimeter leachate after two years is presented in Table 63. These microorganisms should be compared to those in Table 54. The only surviving pathogenic Gram-negative rod is Acinetobacter sp. Several molds did survive, but they were not on the CDC list of hazardous agents (Communicable Disease Center, 1974); however, they are found in pathological lesions, namely Philophora sp., Microsporium sp., and Aspergillus niger (Lennette et al., 1980).

D. FINAL ASSAYS ON DISMANTLED LYSIMETERS

Final assays were made on the six lysimeters which were opened and the contents examined. Two were examined after two years and four were examined after three years. The purpose of this final experiment was to open the remaining lysimeters and to determine the fecal indicator levels and plate counts of the solid waste therein, and to determine the pathogen levels, i.e. Clostridium, Salmonella, and Mycobacterium. An examination of the surface of the waste (not sewage sludge) indicated that the bentonite packed around the edge of the waste and the sides of the lysimeter was intact and as expected kept the water away from the sides of the lysimeter allowing only the center of the deposited solid waste to be watered by the weekly water additions. The exception to this was found in the sewage sludge lysimeter. This lysimeter had no bentonite to direct

TABLE 63. THE IDENTIFICATION OF LEACHATE^a MICROORGANISMS^b TAKEN FROM THE LYSIMETERS

Lysimeter ^c Designation	Microbial Group	Identification ^{a, b} Name	Number of Cultures
UC-A	Bacteria	<u>Acinetobacter</u> ^d sp.	2
		<u>Alcaligenes faecalis</u>	2
		<u>Corynebacterium acquantium</u>	1
		<u>Corynebacterium</u> sp.	1
		<u>Pseudomonas</u> sp.	7
	Fungi	<u>Monosporium</u> sp.	1
		<u>Philophora</u> ^d sp.	1
UC-B	Bacteria	<u>Bacillus</u> sp.	2
		<u>Corynebacterium</u> sp.	4
	Fungi	<u>Aspergillus niger</u> ^d	1
		<u>Monosporium</u> sp.	1
		Yeast	1
UC-C	Fungi	Yeast	1
UC-D	Fungi	<u>Penicillium</u> sp.	1
UC-E	Fungi	Yeast	1
UC-F	None	<u>None</u>	0
		Total	27

a = The leachate samples were taken during the 100th week.

b = Isolates were taken from blood agar, inhibitory mold agar and Sabouraud agar plates were identified by the methods described by Washington (1974, 1980), Weaver et al. (1972), and Koneman et al. (1978).

c = UC-A contains sewage sludge, UC-E sewage sludge and municipal solid waste, UC-C and UC-D, municipal solid waste; UC-E, hospital waste and municipal solid waste, and UC-F, hospital waste.

d = Class Two Agents, Communicable Disease Center (1974), and Lennette et al. (1980).

the water flow. There the sludge had separated at least 1 cm away from the sides of the lysimeter, and it was obvious that the water had run down into a pocket made between the sludge and the lysimeter wall. Judging from the marks made on the wall of the lysimeter the sludge had settled about 3 cm during these past three years. Thus, it appeared that the weekly addition of water ran through the middle of the municipal solid waste in lysimeters UC-B through F, i.e. away from the walls of the cylinder because of the bentonite presence, while in the sewage sludge lysimeter the water ran down the extreme edges of the waste, i.e. next to the cylinder walls because no bentonite had been added to this lysimeter.

The compactness or density of the waste was also examined. This varied with the type of waste. The sewage sludge lysimeter waste appeared to have the consistency of "pudding". It was easy to scoop out the waste during the sampling process. The other waste lysimeters were compacted and all required a lot of "digging" to remove the samples. For the most part, the waste did not appear to have decomposed or changed to any great degree. However, the compacted waste tended to become "soil-like" at 30 cm down from the surface of the waste, i.e. it was "gritty" and was a darker hue when compared to the waste on the surface.

The results of the assays on the solid waste samples contained within the UC-D and UC-F lysimeters at the University of Cincinnati are given in Tables 64 and 65. The fecal indicator levels were higher in the top solid waste layers, taken at 10 cm from the solid waste surface, than in samples taken at lower depths. There would appear to be an association between microbial numbers and pH. The bacterial concentration generally decreased with the log decrease in pH levels. The pH decreased with the waste depth. These results demonstrated that the coliforms had not in fact died as was evident from the low fecal indicator levels in the leachate, but did in fact survive for greater than two years. No other investigator has demonstrated or suggested such a long survival time in the laboratory lysimeters. The higher pH in the lower sections of the solid waste deposited within the lysimeters may perhaps have come from the carbon dioxide given off during the microbial fermentation of the waste materials.

The results of the microbial assays on the leachate, concentrated leachate and lastly the solid waste taken at several levels are presented in Tables 66, 67, 68, and 69. The chemical assays are presented in the lower portion of each table. In all four lysimeters the total coliforms were present in most levels of the solid waste and leachate, but

TABLE 64. MICROBIAL LEVELS OF SOLID WASTE AND LEACHATE FROM A TWO YEAR OLD LYSIMETER^a CONTAINING HOSPITAL WASTE

Quantitative Tests	Lysimeter Contents			Leachate
	Solid Waste at These Descending Levels			
	10 cm	20 cm	30 cm	
<u>MPN/100 g (100 ml)</u>				
Total Coliform ^b	2.2X10 ⁴	9.2X10 ³	1.6X10 ²	<2
Fecal Coliform ^b	20	<20	<20	<2
Fecal Streptococci ^b	3.4X10 ²	1.1X10 ³	70	<2
<u>Agar Plate Counts</u>				
<u>CFU/100 g (100 ml)</u>				
<u>Standard Methods</u>	6.0X10 ⁷	6.0X10 ⁵	4.3X10 ⁴	5.2X10 ⁵
Chocolate (Aerobic)	4.1X10 ⁷	4.8X10 ⁵	8.2X10 ⁴	<1X10 ¹
Chocolate (Anaerobic)	7X10 ⁷	7.1X10 ⁷	8.0X10 ⁶	3X10 ⁵
PEA ^c (Aerobic)	2.1X10 ⁵	2.9X10 ⁵	3.1X10 ³	<1X10 ¹
PEA (Anaerobic)	5.4X10 ⁶	5.8X10 ⁶	1.6X10 ⁵	<1X10 ¹
Blood (Aerobic)	1.7X10 ⁶	1.1X10 ⁶	2.7X10 ⁴	1.2X10 ²
MacConkey (Aerobic)	9.3X10 ⁶	4.2X10 ⁴	6.0X10 ³	<1X10 ¹
MacConkey (Anaerobic)	1.2X10 ⁴	<1X10 ³	<1X10 ³	<1X10 ¹
IMA	4.2X10 ⁵	5X10 ³	1.6X10 ⁴	7.6X10 ²
N-enterococcus	1.2X10 ⁴	<1X10 ³	<1X10 ³	<1X10 ¹
Sabouraud	3.9X10 ⁷	1.7X10 ⁵	1.2X10 ⁵	4.2X10 ²
Tellurite	2.0X10 ⁴	<1X10 ³	<1X10 ³	<1X10 ¹
<u>Chemical Tests</u>				
pH	5.15	5.2	4.95	4.8
Conductivity ^d	1519	874	826	1698
% Moisture	61.5	58.0	61.9	ND ^e

a = The University of Cincinnati Lysimeter F containing hospital waste.

b = Coliforms, Standard Methods (1976) MPN test, Allen et al (1976) and streptococci, Standard Methods (1976) MPN test plus Gram stain for cocci.

c = Phenylethyl alcohol agar without blood.

d = um hos/cm

e = Not determined.

TABLE 65. MICROBIAL LEVELS OF SOLID WASTE AND LEACHATE FROM A TWO YEAR OLD
LYSIMETER^a CONTAINING MUNICIPAL SOLID WASTE

Quantitative Tests	Lysimeter Contents			
	Solid Waste at These Descending Levels			Leachate
	10 cm	20 cm	30 cm	
<u>MPN/100 g (100 ml)</u>				
Total Coliform ^b	1.3X10 ³	2.2X10 ³	20	<2
Fecal Coliform ^b	90	<20	<20	<2
Fecal Streptococci ^b	7.1X10 ⁴	2.4X10 ³	20	<2
<u>Agar Plate Counts</u> <u>CFU/100 g (100 ml)</u>				
Standard Methods	1.0X10 ⁹	2.6X10 ⁸	2.3X10 ⁷	3.1X10 ⁷
Blood Agar (Aerobic)	2.5X10 ⁹	2.3X10 ⁸	4.7X10 ⁷	1.6X10 ⁷
Blood Agar (Anaerobic)	spreader	spreader	spreader	6.5X10 ⁵
PEA ^c (Aerobic)	2.7X10 ⁸	2.2X10 ⁸	4.1X10 ⁷	7.3X10 ⁴
PEA (Anaerobic)	3.5X10 ⁷	1.5X10 ⁷	4.9X10 ⁶	1.5X10 ⁴
MacConkey	<1.0X10 ³	1.0X10 ³	<1X10 ³	<1X10 ³
IMA	4.0X10 ³	2.0X10 ³	<1X10 ³	2.2X10 ⁵
Sabouraud	<1.0X10 ³	<1X10 ³	<1X10 ²	1.0X10 ⁵
Tellurite	3.5X10 ⁷	6.1X10 ⁶	1.7X10 ⁷	2.2X10 ⁴
<u>Chemical Tests</u>				
pH	7.1	6.2	5.15	5.2
Conductivity ^d	1780	1940	2230	4445
% Moisture	69.65	65.34	64.09	ND ^e

a = The University of Cincinnati Lysimeter D containing municipal waste.

b = Coliforms, Standard Methods (1976) MPN test, Allen et al (1976) and streptococci, Standard Methods (1976) MPN test plus Gram stain for cocci.

c = Phenylethyl alcohol without blood.

d = um hos/cm

e = Not determined.

TABLE 66. FINAL MICROBIAL ANALYSIS OF LEACHATE AND SOLID WASTE FROM LYSIMETER CONTAINING SEWAGE SLUDGE, UC-A AFTER THREE YEARS

Microbial Tests	Microbial Levels CFU or MPN/100 g (ml)				Leachate
	Solid Waste				
	Distances Below Solid Waste Surface (cm)				
	10	20	30	40	
<u>Fecal Indicator MPN Tests (MPN/100 g [ml])</u>					
Total Coliform ^a	2,400	< 20	< 20	110	4
Fecal Coliform ^a	< 20	< 20	< 20	< 20	< 20
Fecal Streptococci ^b	< 20	< 20	< 20	< 20	24
<u>Clostridium</u>					
<u>perfringens</u> ^c	2.4X10 ⁷	2.4X10 ⁷	2.4X10 ⁷	2.4X10 ⁷	.3.5X10 ^{3d} 230
<u>Agar Plate Counts CFU/100 g ml</u>					
EMB ^e	1.0X10 ⁹	1X10 ⁵	1X10 ⁵	7X10 ⁴	1x10 ⁶
MacConkey	> 10 ⁶	2X10 ²	6X10 ²	8X10 ²	2.5x10 ⁵
m-enterococcus	< 10 ²	< 10 ²	< 10 ²	< 10 ²	40
TSC ^f (Anaerobic)	9.8X10 ⁴	2.4X10 ⁵	3.2X10 ⁵	2.5X10 ⁵	1 ^d
TSC (Aerobic)	1X10 ³	< 1X10 ³	< 1X10 ³	< 1X10 ³	< 1X10 ^{3ad}
M7X10 ⁹	< 10 ³	< 10 ³	< 10 ³	< 10 ³	< 10 ^{3d}
Mycosal	3.6X10 ⁴	< 10 ²	< 10 ²	< 10 ²	< 10
IMA ^h	> 10 ⁵	3X10 ²	9X10 ²	9X10 ²	2.5X10 ²
pH	6.0	6.7	6.8	6.8	7.1
Conductivity ⁱ	464	490	452	477	1962
% Moisture	81.0	75.9	75.6	66.6	ND ^j

a = Standard Methods (1976) most-probable-number (MPN) test adapted by Allen et al., (1976).

b = Standard Methods (1976) most-probable-number (MPN) test adapted by including those positive tubes only which contain Gram-positive cocci.

c = Iron milk stormy fermentation tubes.

d = Concentrated leachate.

g = Middlebrook 7H10 agar.

e = Eosin methylene blue.

h = Inhibitory mold agar.

f = Tryptone sulfite cycloserine agar.

i = umhos/cm.

j = Not determined.

TABLE 67. FINAL MICROBIAL ASSAYS OF LEACHATE AND SOLID WASTE FROM LYSIMETER CONTAINING SEWAGE SLUDGE AND MUNICIPAL SOLID WASTE (UC-B)

Microbial Tests	Microbial Levels CFF or MPN/100 g (ml)			Leachate
	Solid Waste			
	Distances Below Solid Surface in cm			
	10	20	30	
<u>Fecal Indicator MPN/100 g (ml)</u>				
Total Coliform ^a	110	170	33	< 2
Fecal Coliform ^a	< 2	< 2	< 20	< 2
Fecal Streptococci ^b	< 2	200	1.52X10 ³	< 2
<u>Clostridium</u>				
perfringens ^c	5.4X10 ⁶	5.4X10 ⁶	9.2X10 ⁶	54 ^d
<u>Agar Plate Counts CFU/100 g ml</u>				
EMB ^e	5.1X10 ⁸	5.0X10 ⁸	3.6X10 ⁸	4.0X10 ⁷
MacConkey	< 1X10 ⁴	< 1X10 ⁴	< 1X10 ⁴	5.0X10 ³
M-enterococcus	< 1X10 ⁴	2.1X10 ⁶	9.0X10 ⁵	< 1X10 ³
TSC ^f (Anaerobic)	1.6X10 ⁸	1.2X10 ⁷	9.5X10 ⁶	< 10 ^d
TSC ^f (Aerobic)	< 1X10 ⁵	< 1X10 ⁵	< 1X10 ³	< 1 ^{ad}
M7H10 ^g	< 1X10 ⁵	< 1.10 ⁵	< 1X10 ³	< 1 ^d
<u>Chemical Parameters</u>				
pH	5.4	4.9	5.9	5.6
Conductivity ^h	635.8	635.8	63	1956
% Moisture	71	70	69	99

a = Standard Methods (1976) most-probable-number (MPN) test adapted by Allen et al., (1976).

b = Standard Methods (1976) most-probable-number (MPN) test adapted by those including positive tubes only which contain Gram-positive cocci.

c = Iron milk stormy fermentation tubes.

f = Tryptone sulfite cycloserine agar.

d = Concentrated leachate.

g = Middlebrook 7H10 agar.

e = Eosin methylene blue.

h = umhos/cm.

TABLE 68. THE FINAL MICROBIAL LEVELS UC-C MUNICIPAL SOLID WASTE LYSIMETER

Microbial Tests	Microbial Levels, CFU or MPN/100 g (ml)			Leachate
	Solid Waste Depth (cm)			
	10	20	30	
<u>Fecal Indicator MPN/100 g (ml)</u>				
Total Coliform ^a	230	80 ^c	< 20	< 20
Fecal Coliform ^a	< 20	< 20	< 20	< 20
Fecal Streptococci ^b	70	130	< 20	< 20
<u>Clostridium</u>				
perfringens ^c	>2.4X10 ⁵	9.2X10 ⁴	>2.4X10 ⁵	24 ^d
in Iron Milk				
<u>Plate Counts CFU/100 g (ml)</u>				
EMS ^e	1.5X10 ⁷	2.0X10 ⁷	1.5X10 ⁷	1.6X10 ⁷
MacConkey	1.7X10 ⁵	3.0X10 ⁴	6X10 ⁴	1X10 ⁵
M- <i>enterococcus</i>	< 10 ⁴	< 10 ⁴	< 10 ⁴	< 1X10 ⁴
TSC ^f (Anaerobic)	4X10 ⁶	4X10 ⁶	1X10 ⁶	120 ^{ad}
TSC ^f (Aerobic)	< 1X10 ⁴	< 1X10 ⁴	< 1X10 ⁴	< 1 ^{ad}
M7H10 ^g	< 10 ⁴	< 10 ⁴	< 10 ⁴	< 10
<u>Chemical Parameters</u>				
pH	5.9	5.4	4.9	4.9
Conductivity ^h	31	31	41	636
% Moisture	70	72	71	ND ⁱ

a = Standard Methods (1976) most-probable-number (MPN) test adapted by Allen, et al., (1976).

b = Streptococci using Standard Methods (1976) MPN test plus Gram stain for cocci.

c = Iron milk stormy fermentation tubes.

d = Concentrated leachate.

g = Middlebrook 7H10 agar.

e = Eosin methylene blue.

h = umhos/cm.

f = Tryptone sulfite cycloserine agar. i = Not determined.

Reproduced from
best available copy.



TABLE 69. FINAL MICROBIAL ASSAYS OF LEACHATE AND SOLID WASTE FROM LYSIMETER UC-2 CONTAINING A MIXTURE OF HOSPITAL AND MUNICIPAL SOLID WASTE

Microbial Tests	Microbial, CFU or MPN/100 g (ml)			Leachate
	Solid Waste Depth (cm)			
	Distances Below Waste Surface			
	10	20	30	
<u>Fecal Indicator MPN/100 g (ml)</u>				
Total Coliform ^a	130	80	70	< 2
Fecal Coliform ^a	< 20	< 20	< 20	< 2
Fecal Streptococci ^b	80	20	330	2
<u>Clostridium</u>				
perfringens ^c	3.5X10 ⁵	2.4X10 ⁵	2.2X10 ⁶	110 ^d 700
<u>Plate Counts CFU/100 g (ml)</u>				
EMB ^e	6.3X10 ⁶	4.4X10 ⁶	1.7X10 ⁷	4.25X10 ⁶
MacConkey	< 10 ²	< 10 ²	< 10 ²	5X10 ²
M-enterococcus	< 10 ²	< 10 ²	< 10 ²	< 10 ²
TSC (Anaerobic) ^f	4.9X10 ⁴	2.8X10 ⁵	3.1X10 ⁵	3 ^d
TSC (Aerobic) ^f	< 10 ²	< 10 ²	< 10 ²	< 1
M7H10 ^g	< 10 ³	< 10 ³	< 10 ³	< 10
Sabouraud	< 10 ⁴	< 10 ⁴	< 1X10 ⁴	< 1X10 ⁴
<u>Chemical Parameters</u>				
pH	5.2	5.2	5.7	5.7
Conductivity ^h	157	117	176	1086
% Moisture	68.6	69.8	69.9	99.6

a = Standard Methods (1976) most-probable-number (MPN) test adapted by Allen et al., (1976), completed and confirmed, Standard Methods (1976) tests.

b = Standard Methods (1976) most-probable-number (MPN) test adapted by including those positive tubes only which contain Gram-positive cocci.

c = Iron milk stormy fermentation tubes. f = Tryptone sulfite cycloserine agar.

d = Concentrated leachate.

g = Middlebrook 7H10 agar.

e = Eosin methylene blue.

h = umhos/cm.

the fecal coliforms were absent from all samples. The fecal streptococci numbers were generally low or nonexistent in all lysimeters except for one level from the UC-B lysimeter in which the m-enterococcus agar plate counts indicated the presence of $10^4/100$ ml. These results demonstrate the survival of a few total coliforms and streptococci for three years in these laboratory lysimeters.

The typtone sulfite cycloserine (TSC) agar plate counts were consistent for all four lysimeters and demonstrated that all levels of the solid waste had roughly 10^5 to 10^6 and the leachate had < 1 to 20 CFU/100 g (ml) of Clostridium perfringens. The iron milk most-probable number (MPN) results on the other hand gave solid waste levels varying from 10^4 to 10^7 and leachate levels varying from 10^1 to $10^2/100$ g (ml) using concentrated leachate, and varying from 10^2 to 10^3 MPN/100 ml using nonconcentrated leachate, indicating that some cells were lost during the process. From these results it is easy to see why the C. perfringens was not cultured previously in nonconcentrated leachate, i.e. as they were present in numbers which would only occasionally grow out on the anaerobic blood agar plate. Thus the TSC and the MPN enumerative procedures for the solid waste results were close and appeared to support one another except for the leachate tests. There the iron milk

MPN levels were sometimes 10^2 times greater than the TSC plate counts.

The concentrated leachate was cultured for mycobacteria, however, none were found. Fungal cultures were not carried out on UC-B and UC-C. Few if any molds were found on UC-E, however, levels of 10^1 and $10^3/100$ g were found in the UC-A samples.

Three chemical parameters were determined, the conductivity, percent moisture, and pH. The high moisture levels (81%) and the high pH (up to pH 7.1) as seen in the sewage sludge may have been a factor in the high bacterial survival level demonstrated by the total coliforms and the Clostridium perfringens.

The microorganisms identified from the solid waste and leachate are presented in Tables 70, 71, and 72.

Thus, the presumptive identification of other clostridia species include C. botulinum, C. limosum, C. subterminal, C. tetani, C. bifermentans, C. butyricum and C. sporogenes. Of these clostridial species, C. perfringens, C. botulinum, and C. tetani are all capable of causing serious illness which on occasion could be terminal. The most commonly involved bacterium was C. perfringens. It is found in wound infection (gas gangrene), uterine infection, bacteremia, unusual localized infections, such as pneumonia and meningitis, and food poisoning (Davis et al., 1980). C.

TABLE 70. BACTERIA ISOLATED FROM THREE YEAR OLD SEWAGE SLUDGE LYSIMETER

Isolate	Identification Procedure	Number of Strains
<u>Acinetobacter calcoaceticus</u> var. <u>lwoffii</u> ^a	API-20E	5
<u>Aeromonas hydrophila</u>	API-20E	1
<u>Alcaligenes</u> sp.	King-Weaver ^b	4
<u>Clostridium botulinum</u> (possible) ^{c, d}	API-20A	1
<u>Clostridium linozum</u> (possible) ^c	API-20A	1
<u>Clostridium perfringens</u>	API-20A	4
	Washington (1981)	15
<u>Clostridium subterminale</u> (possible) ^c	API-20A	1
<u>Clostridium tetani</u> (presumptive) ^{d, a}	API-20A	1
<u>Clostridium</u> sp.	Washington (1981)	4
<u>Pleisomonas</u> sp.	Bergey's Manual	1
<u>Pseudomonas</u> sp.	King-Weaver ^b	9
<u>Pseudomonas</u> sp. (Fluorescent Group)	API-20E	1
<u>Serratia fonticola</u>		1
	Total	49

a = Listed as Class 2 Agent, Communicable Disease Center (1974).

b = Adapted from Weaver (1972) and from Koneman (1979).

c = Further tests are needed for complete identification.

d = Complete identification would require a mouse protection test
(D. S. Smith, Dowell, and Allen, 1980).

TABLE 71. BACTERIA ISOLATED FROM A MIXED WASTE LYSIMETER (UC-B) CONTAINING MUNICIPAL SOLID WASTE AND SEWAGE SLUDGE

Isolate	Identification Procedure	Number of Strains
<u>Acinetobacter calcoaceticus</u> var. <u>lwoffii</u> ^a	API-20E	5
<u>Acinetobacter</u> sp.	King-Weaver ^b	1
<u>Citrobacter amalonaticus</u>	API-20E	1
<u>Citrobacter freundii</u>	API-20E	1
<u>Clostridium bifermentans</u>	API-20A	1
<u>Clostridium botulinum</u> (presumptive) ^{c, a}	API-20A	1
<u>Clostridium butyricum</u>	API-20A	1
<u>Clostridium perfringens</u>	API-20A	1
	Washington (1981)	6
<u>Clostridium ramosum</u>	API-20A	1
<u>Clostridium sporogenes</u>	API-20A	1
<u>Clostridium</u> sp.	Washington (1981)	1
<u>Enterobacter cloacae</u>	API-20E	1
<u>Escherichia coli</u>	Edwards & Ewing ^d	1
<u>Pseudomonas</u> sp.	King-Weaver ^b	1
	Total	20

a = Listed as a Class 2 Agent, Communicable Disease Center (1974)

b = From Koneman et al. (1979) and Weaver (1972).

c = Complete identification would require a mouse protection test
(D. S. Smith, Dowell, and Allen, 1980).

d = Edwards and Ewing (1962).

TABLE 72. MICROORGANISMS ISOLATED FROM A MUNICIPAL SOLID WASTE LYSIMETER (UC-C) AND MUNICIPAL WASTE WITH HOSPITAL WASTE (UC-E)

Isolate	Identification Procedure	Number of Strains
UC-C		
Bacteria:		
<u>Bacillus</u> sp.	Bergy's Manual	1
<u>Citrobacter amalonaticus</u>	API-20E	2
<u>Citrobacter freundii</u>	API-20E	1
<u>Clostridium perfringens</u>	API-20A	1
	Washington (1981)	7
<u>Enterobacter agglomerans</u>	API-20E	1
<u>Klebsiella oxytoca</u>	API-20E	2
<u>Pseudomonas</u> sp.	Weaver et al. (1972)	4
	Koneman et al. (1979)	
Fungi:		
<u>Fusarium</u> sp.	Koneman (1978)	1
<u>Monosporium apiospermum</u>	Koneman (1978)	2
<u>Penicillium</u> sp.	Koneman (1978)	3
<u>Phoma</u> sp.	Koneman (1978)	1
<u>Pleospora</u> sp.	Koneman (1978)	2
UC-E		
<u>Clostridium perfringens</u>	Washington (1981)	9
	Total	37

a = Listed as a Class 2 Agent, Communicable Disease Center (1974).

b = Adapted from Weaver (1972) and Koneman et al. (1979).

botulinum produces a toxin which when ingested produces botulism. C. tetani produces tetanus, an acute and almost always fatal disease. These bacteria are usually found in soil. However, all three produce potent toxins which could drain from the landfill in the leachate. If large amounts of toxins were produced, which drained into ground water used for drinking or bathing purposes, then they could produce a public health problem. Also, the effect on farm animals drinking this water would be uncertain or perhaps detrimental. Other pathogens from these lysimeters were Klebsiella oxytoca, Monosporium apiospermum, and Acinetobacter calcoaceticus var. lwoffi. These are of low virulence. They are various opportunistic pathogens, but can produce severe infections.

E. CONCLUSIONS

1. The Boone County and the Center Hill leachate assays for the fecal indicator levels at two to six years were so low and the pathogens so few in number that it was unlikely that any public health problems would be brought about from exposure to leachate. The pathogens found were not particularly infections, but were opportunistic pathogens. In addition the results indicated that the fecal streptococci were decreasing in number year by year.

2. Similar conclusions can be made for the six lysimeters at the University of Cincinnati, except for the

anaerobes. C. perfringens (10^5 MPN/100 g) was present in the solid waste from within the lysimeters. Also, C. botulinum and C. tetani were isolated from these wastes. These latter microorganisms produce some of the world's most potent toxins. We might speculate that these bacteria attached to the solid waste within the landfills may produce toxins which could travel a mile or so in the ground water. In addition, the S. faecalis survival for over two years indicated Gram-positive bacterial resistance for at least that length of time. Finally, the Clostridium lasted for three years. If the waste was kept anaerobic, then they could perhaps last even longer.

3. There was additional evidence that these lysimeters could contain viable bacteria for over two years.

a. When leachate samples were sampled daily instead of weekly, total coliform, fecal coliform, and fecal streptococci were measured in numbers not seen since the first few weeks following lysimeter construction. Evidently these fecal indicator bacteria after draining through the waste in the leachate died in the storage area in the bottom of the lysimeters, but when samples were taken on a daily basis they apparently were still alive.

b. All three fecal indicator bacteria survived in solid waste taken from lysimeters two years old.

c. In two lysimeters total coliforms were found in leachate samples taken at the 100th week.

CHAPTER VII

RESULTS AND DISCUSSION: COMMERCIAL FULL-SCALE AND EXPERIMENTAL FULL-SCALE LANDFILL ASSAYS

A. OVERVIEW

Two types of samples were examined here: (1), the leachate from full-scale landfills taken over a period of several years, and (2), both leachate and solid waste taken from within a landfill when the experiment was ended (closure) and the final assays were to be made.

B. LEACHATE ANALYSIS

1. Microbial Enumeration and Identification

Leachate samples were examined for fecal indicator levels and plate counts. The results are shown in Tables 73 and 74. The fecal indicator levels, i.e. the total coliform, fecal coliform, and the fecal streptococci from the Boone county leachate were usually 10^2 MPN/100 ml or less (Table 73). The coliforms had apparently died out while the streptococci were present on an inconsistent basis. The Boone County landfill at this time was six years old when these determinations were made.

On the other hand the commercial landfill had fecal indicator levels ranging from 10^2 to 10^4 MPN/100 ml of leachate. These levels were higher perhaps because this was an active landfill and received waste daily. Later we found

TABLE 73. FECAL INDICATOR LEVELS IN BOONE COUNTY^a AND COMMERCIAL LANDFILL LEACHATE

Landfill Sites	Sampling Date	Age of Waste (yr)	Fecal Indicator Levels (MPN/100 ml)		
			Total Coliforms	Fecal Coliforms	Fecal Streptococci
Boone County Landfill	01/16/77	6	8	< 2	< 2
TC-1	01/24/78	7	ND ^b	ND	200
	02/07/78	7	ND	ND	230
	03/02/78	7	ND	ND	20
	04/11/78	7	ND	ND	50
	05/30/78	7	< 30	< 30	230
	11/06/78	7	23	< 2	20
Commercial Landfill					
CL-4 ^c	11/15/77	7	< 2	< 2	2x10 ²
CL-4 ^c	06/05/78	8	< 30	< 30	20
CL-3 ^d	07/24/79	d	1.8x10 ⁴	23	3.5x10 ⁴
CL-1 ^d	11/01/79	d	ND	ND	5.4x10 ⁴
CL-1 ^d	04/ /80	d	ND	ND	2.7x10 ³

a = The Boone County landfill was six years old.

b = Not determined.

c = Leachate was taken from waste that was seven years old.

d = Sample was taken from a spring of leachate emerging from the side of the landfill.

TABLE 74. MICROBIAL COUNTS^a FROM LANDFILL LEACHATE

Quantitative Tests	LANDFILLS (LYSIMETERS)		
	Boone County	Commercial Landfill	
	TC-1 (6 years) ^d	CL-4 ^b (7 yr) ^d	CL-1 through 3 ^c (7 wk) ^d
<u>MPN/100 ml</u>			
Total Coliforms	< 30	< 30	1.8X10 ⁴
Fecal Coliforms	< 30	< 30	23
Fecal Streptococci	1X10 ²	50	3.5X10 ⁴
<u>Agar Plate Counts, CFU/100 ml</u>			
Blood-aerobic	7.3X10 ⁵	5.0X10 ⁵	6.2X10 ⁶
Blood-anaerobic	8.0X10 ⁵	1.9X10 ⁵	1.3X10 ⁵
Brain heart	4.0X10 ⁵	1.8X10 ⁴	2.7X10 ⁶
Eosin Methylene Blue	3.0X10 ⁵	6.0X10 ³	5.0X10 ⁶
Inhibitory Mold ^e	1.7X10 ⁴	< 10 ³	< 10 ³
KP Streptococcal	< 10 ³	5.0X10 ²	2.5X10 ³
Mycosel ^e	2.0X10 ³	< 10 ³	< 10 ³
Sabouraud ^e	4.0X10 ⁵	3.0X10 ⁵	< 10 ³
Tellurite	5.0X10 ⁴	5.0X10 ²	< 10 ³

a = These are the averages of two or more leachate samples.

b = The leachate came from pipes deep within the landfill ("old" leachate).

c = The leachate emerged from the soil at many sites ("fresh" leachate).

d = Age of waste in landfills and lysimeters.

e = Incubated at ambient temperature.

that the CL-4 leachate levels decreased steadily while those from the three leachate springs, sites CL-1, CL-2, and CL-3, had higher total coliform, fecal coliform, and fecal streptococci levels. The main difference, apparently, was that the CL-4 leachate came from a ten year old waste site via a pipe placed deep within that waste. No new waste had been placed on top of it since it was deposited. On the other hand CL-1, CL-2, and CL-3 leachate samples apparently came from the newly (daily) deposited waste placed on top of the landfill which drained downward and emerged from shallow surface leachate springs. Also, the fecal coliform levels were about 10^4 MPN/100 ml and thus indicated recent waste deposits.

The plate counts are shown in Table 74. There are one series of the Boone County TC-1 leachate measurements and two from the commercial landfill, i.e. from the older waste and from the newer waste described above. The Boone County leachate microorganisms had higher fungal and Gram-positive rod counts and thus were similar to the older commercial landfill leachate levels. On the other hand the distinguishing marks of the newer leachate (commercial landfill) were the low levels of Gram-positive rods and fungi along with higher streptococcal levels as determined by the KF streptococcal plates. Finally, the aerobic blood

agar plates had higher counts than the anaerobic plates, indicating few anaerobes were present.

The initial isolation of pathogens from landfill leachate is presented in Table 75. A second effort a year later is presented in Table 76. All microbial isolations and identifications from leachate were made for the purpose of finding the surviving pathogens in landfills. The identified pathogens are marked (Communicable Disease Center, 1974; Davis et al., 1980; and Lennette et al., 1974, 1980). The pathogens from the Boone County site include Listeria monocytogenes, Moraxella and Allescheria boydii. The streptococci isolated from the same site included those which may be found in human feces and fowl stools. No pathogens were isolated from the commercial landfill (Table 75), but fecal bacteria were found such as the anaerobic streptococci, the alpha streptococci, and the Clostridium. In Table 76 none of the isolates appeared to be highly infectious (e.g., Salmonella) except perhaps for the Mycobacterium. There were several Enterobacteriaceae not seen previously such as Enterobacter, Providencia, Serratia, Proteus, and Citrobacter.

Thus we may conclude the following: (1) Large numbers of Gram-negative rods were present in the active

TABLE 75. PARTIAL IDENTIFICATION OF MICROORGANISM ISOLATED FROM SPECIFIC LEACHATE SAMPLES IN SPRING 1978

<u>Microorganisms Identified From Landfills:</u>	
<u>Boone County, Kentucky</u> <u>Landfill TC-1</u>	<u>Commerical Landfill^d</u> <u>Cincinnati, Ohio</u>
<u>Aeromonas</u> sp.	<u>Achromobacter</u> sp.
<u>Allescheria boydii^a</u>	<u>Anaerobic Streptococcus</u>
<u>Bacillus</u> sp.	<u>Bacillus</u> sp.
<u>Corynebacterium</u> sp.	<u>Clostridium fallax</u>
<u>Listeria monocytogenes^b</u>	<u>Clostridium</u> sp.
<u>Moraxella</u> sp. ^b	<u>Corynebacterium</u> sp.
<u>Neisseria</u> sp.	<u>Pseudomonas</u> sp.
<u>Serratia marcescens</u>	<u>Streptococcus</u> sp.
<u>Streptococcus</u> , sp.	<u>Streptococcus</u> , Alpha
<u>Streptococcus faecalis</u>	
<u>Streptococcus durans</u>	
<u>Streptococcus faecium</u>	
<u>Streptococcus</u> O	
<u>Streptococcus</u> , Alpha	
Yeasts ^c	

a = While not on the Communicable Disease Center (1974) list of the hazardous etiologic agents, it is considered to be pathogenic (Lennette et al., 1980)

b = Listed in the Communicable Disease Center (1974) Classification of Etiologic Agents on the Basis of Hazard, class 2.

c = Yeasts were not identified.

d = Name withheld since commercial firm involved.

TABLE 76. PRESUMPTIVE IDENTIFICATION OF BACTERIA FROM
COMMERCIAL LANDFILL LEACHATES

Date Leachate Sample Taken	Microbial Identification ^a	Number of Isolates
05/15/79	<u>Acinetobacter</u> sp. ^b	1
	<u>Aeromonas</u> sp.	11
	<u>Bacillus</u> sp.	9
	<u>Citrobacter</u> sp.	6
	<u>Diptheroids</u>	6
	<u>Enterobacter</u> sp.	2
	<u>Flavobacterium</u> sp.	2
	<u>Moraxella</u> sp. ^b	2
	<u>Proteus</u> sp.	2
	<u>Providencia</u> sp. ^b	3
	<u>Pseudomonas</u> sp.	8
	<u>Serratia</u> sp.	1
	<u>Streptococcus</u> sp.	14
	<u>Vibrio</u> sp.	2
	<u>Xanthomonas</u> sp.	1
	Subtotal	70
06/12/79	<u>Mycobacterium</u> sp. ^b	2
07/23/79	<u>Acinetobacter</u> sp. ^b	1
	<u>Aeromonas</u> sp.	4
	<u>Citrobacter</u> sp.	2
	<u>Diptheroids</u>	1
	<u>Proteus</u> <u>morganii</u>	7
	<u>Pseudomonas</u> sp.	1
	<u>Serratia</u> <u>marcescens</u>	1
	<u>Streptococcus</u> sp.	1
	Subtotal	20
	TOTAL	90

a = Isolated on blood agar, EMB, a Lowenstein-Jensen slants, and identified by the method of Weaver, et al., (1972); Koneman et al., (1979); and Edwards and Ewing (1962).

b = Pathogens as determined by the Communicable Disease Center (1974). Classification of Etiological Agents on the Basis of Hazard, class 2.

commercial landfill as indicated by the growth on the eosin methylene blue (EMB) plates, the bacterial identification, and by the levels of the total and the fecal coliforms. Yet no Salmonella isolates were found. (2) Forty-eight hours of growth on anaerobic blood agar plates generally did not yield Clostridium perfringens. (3) In the commercial landfill the fungal and Gram-positive nonsporeforming rods were lower (10^2 to 10^4 MPN/100 ml of leachate) than the counts on other media (10^3 to 10^6 CFU/100 ml of leachate). Perhaps the Gram-negative rods overgrew the fungi and the other slower growing microorganisms. (4) The fecal streptococci apparently lingered on at low levels in the landfill for two to seven years, suggesting that pathogens of equal resistance could also do the same. (5) Several pathogenic bacteria and molds have been found in leachate from landfills. These include Listeria monocytogenes, Acinetobacter, Moraxella, Allescheria boydii, and Mycobacterium.

2. The Identification of Streptococcal Groups and Species

The results of the streptococcal identification by the biochemical grouping method of Facklam et al., (1974) and the biochemical species determinations for Group D strains by the method of Pavlova et al., (1972) and Bordner et al., (1978) are presented in Table 77. The source of streptococci for these determinations was the commercial

TABLE 77. THE PRESUMPTIVE IDENTIFICATION OF STREPTOCOCCAL GROUPS^a AND SPECIES^b ISOLATED FROM LANDFILL LEACHATES

Streptococcal Sources	Grouping Parameters ^a				Number	Isolates Identification ^b
	Sensitivity to Bacitracin	Nippurate Test	Growth On:			
			Bile Esculin	6.5% NaCl		
<u>Commercial Landfill Leachates:</u>						
Sample 1	- ^d	-	+	+	16	D-enterococcus (15 <u>S. faecalis</u>)
Sample 2	+	-	-	+	2	D-enterococcus (2 <u>S. faecalis</u>)
	-	-	-	+	2	D-enterococcus
	+	-	-	-	3	Viridans ^f
	-	-	-	-	11	D-enterococcus
Sample 3	-	-	-	+	5	D-enterococcus
	-	-	-	-	2	Lactic Streptococci ^f
Sample 4	-	-	-	-	1	Lactic Streptococci ^f
	-	-	-	-	2	Viridans ^f
Sample 5	-	-	+	-	2	D-nonenterococcus
	-	-	-	+	2	D-enterococcus
	-	-	+	+	4	D-enterococcus
<u>Streptococcus faecalis</u> ATCC 19433	-	-	+	+	1	D-enterococcus
TOTAL 91						

a = Facklam et al., (1974)

d = Negative.

e = Positive.

b = Pavlova et al., (1972), Standard Methods (1976), Bordner et al., (1978) for the species determinations.

f = Bergy's Manual (1975)

c = Obtained from the surface of the landfill slope.

Wilson and Miles (1975)

Reproduced from
best available copy.



landfill. The commercial landfill with the recently added waste (within the past year) contained a variety of cocci all of which had human and animal fecal origins. Both Streptococcus faecalis and the viridans streptococci were identified. The Facklam streptococcal grouping process placed these streptococci into four main groups: D enterococcus, derived from human and animal fecal material; D nonenterococcus, derived from animal fecal material; viridans, from humans and animals; and lactic streptococci from milk products (animals) (Facklam et al., 1974; R. E. Buchanan et al., 1974; and Wilson and Miles, 1975). These isolates were compared with Table 26 of Chapter 4 on streptococcal origins. This table states that the identified species, S. faecalis, is of human and animal origin.

Seventeen of these streptococcal strains were also grouped with Streptex antisera (Wellcome Reagents, Ltd., Backingham, England). The results are presented in Table 78. The Group D-enterococcus identifications (Table 78) were confirmed by the agglutination tests. Furthermore, the strains classified as S. viridans, that carried both Group B and G antigens were probably Group B or Group G. Group B streptococci originates from man and cattle, Group G originates from man (Wilson and Miles, 1975). This suggests further the human origin of the strains. The last three strains were also classified as S.

TABLE 78. STREPTOCOCCI GROUPED BY STREPTEX ANTISERA

Leachate Source	Positive Agglutination Reaction of Lancefield Streptococcal Groups Using Streptex ^a Antisera						No. of Strains
	A	B	C	D	E	G	
<u>Commercial Landfill</u>							
<u>Leachates^b</u>							
Sample 1	- ^c	-	-	+ ^d	-	+	1 ^e
Sample 2	-	+	-	-	-	+	1 ^f
Samples 1,2	-	-	-	+	-	-	6 ^e
Sample 1	-	-	-	-	-	-	3 ^f
<u>Streptococcus</u> <u>faecalis</u> ATCC #19433	-	-	-	+	-	-	<u>1</u>
Total Strains Tested							11

a = Welcome Research Laboratories, Beckenham, England BR3 3BS.

b = From Commercial Landfill sites CL-1 and CL-2, respectively.

c = No Agglutination.

d = Agglutination.

e = Labeled D-enterococcus by the Facklam et al., (1974) method.

f = Labeled viridans by the Facklam et al., (1974) method.

viridans and carried no D but B and G grouping antigens. Thus, the Facklam et al., (1974) grouping procedure is confirmed for its accuracy.

The identification of groups and species of streptococci indicated both human and animal fecal material was present in the landfills. The Streptococcus faecalis originating from both man and animal feces was present in the commercial landfill. In addition, this landfill had a great variety of streptococci, namely D-nonenterococcus, lactic streptococci, D-enterococcus, and viridans. The latter two microorganisms are usually found in man while the others are found in animals.

In conclusion, the significance of the streptococcal survival with the variety of groups and species present, demonstrated that human streptococci can survive in human waste. These bacteria survived the environmental stresses and the leachate toxicity and were therefore resistant forms. Finally, the survival of the resistant streptococci suggests that other resistant genera will also be present, such as Mycobacterium and Clostridium.

3. Bacterial Isolations Using Improved Methods

a. Isolation Results

Improved methods were used to culture Salmonella, Mycobacterium, and Clostridium (Table 79). A great effort was made to find the Salmonella in the leachate. In the

TABLE 79. MICROBIAL ISOLATIONS FROM THE COMMERCIAL LANDFILL

Sampling Date	Leachate Sample	Microorganisms Isolated		
		<u>Salmonella</u>	<u>Clostridium</u>	<u>Mycobacterium</u>
06/06/80	1	+ ^a	ND ^b	+
	2	+	ND	+
	3	+	ND	+
06/16/80	4	- ^c	ND	+
	5	-	ND	+
	6	-	ND	+
06/23/80	7	-	-	+
	8	-	-	+
	9	-	+	+
	10	-	-	+
	11	-	-	+

a = Positive Results

b = Not Determined

c = Negative Results

past all kinds of stress-reducing procedures were used without success. This time multiple procedures and various kinds of media were used. Finally, Salmonella were found in all three leachates samples on the 9th of June, but none were found afterwards. Mycobacterium was found in all samples by using concentrated and digested leachate preparations. By also using concentrated leachate samples, but without digestion C. perfringens was isolated from iron milk medium. Only the third week samples were inoculated into iron milk medium. Stormy fermentation occurred in all three samples, but C. perfringens was isolated only from sample 9. Accompanying assays made on the same leachate samples are presented in Table 80 and 81.

The identified microorganisms from these same eleven leachates are presented in Table 82. The important or significant bacteria to public health are marked with an asterisk to underline their risk, i.e. they are Class 2 agents, as determined by the Communicable Disease Center (1979) etiologic agent list. The Salmonella can produce gastroenteritis, septicemia, and enteric fever. The Acinetobacter is capable of producing severe primary infections, such as meningitis, and septicemia. The Mycobacterium produce a large variety of diseases such as tuberculosis, chronic cavity lung disease, lymphadenitis in children, bone and joint infection, and skin and soft tissue diseases. While

Table 80. FECAL INDICATOR BACTERIA ISOLATED FROM LANDFILL LEACHATE

Sampling Date	Leachate Sample	Fecal Indicator MPN/100 ml		
		Total Coliforms ^a	Fecal Coliforms ^a	Fecal Streptococci ^b
06/09/80	1	ND ^c	ND	ND
	2	ND	ND	ND
	3	ND	ND	ND
06/16/80	4	3.5X10 ³	1.7X10 ³	4.5X10 ⁴
	5	9.0X10 ²	79	4.0X10 ⁴
	6	9.2X10 ²	49	1.0X10 ⁵
06/23/80	7	4.6X10 ²	< 2	4.8X10 ³
	8	3.5X10 ²	2	4.9X10 ³
	9	9.2X10 ²	70	1.1X10 ⁴
	10	13	< 2	< 1.0X10 ³
	11	1.7X10 ²	9	3.0X10 ²

a = Standard Methods (1976) modified by Allen et al., (1976).

b = Standard Methods (1976) modified by using the Gram stain to confirm the presence of cocci.

c = Not determined.

TABLE 81. LEACHATE PARAMETERS

Sampling Date	Leachate Sample Number	Sampling Site Indicated	Temperature of Leachate at Landfill C	Leachate pH
06/09/80	1	CL-1 ^a	28	7.4
	2	CL-2	28	7.35
	3	CL-3	28	7.4
06/16/80	4	CL-1	28	7.4
	5	CL-2	28	7.4
	6	CL-3	28	7.4
06/23/80	7	CL-1	28	7.8
	8	CL-2	28	7.8
	9	CL-3	28	7.6
	10	CL-4 ^d	22	7.0
	11	CL-5 ^e	21	6.9

a = Commercial landfill sites CL-1, CL-2, and CL-3 came from leachate springs at the northeast slope of the landfill, and sites CL-4 and CL-5 came from pipes extending from waste placed within the landfill in 1970.

TABLE 82. MICROORGANISMS ISOLATED FROM COMMERCIAL LANDFILL
LEACHATE (PART I)

Leachate Sample	Microorganisms Identified	Number of Microbial Isolates Found
1	<u>Acinetobacter</u> ^a sp.	5
	<u>Enterobacter agglomerans</u>	2
	<u>Enterobacter cloacae</u>	1
	<u>Enterobacter</u> sp.	1
	<u>Mycobacterium</u> ^a	2
	<u>Providencia alcalifaciens</u>	1
	<u>Providencia</u> sp.	1
	<u>Pseudomonas</u> sp.	5
	<u>Salmonella</u> ^a sp.	2
2	<u>Acinetobacter</u> ^a sp.	3
	<u>Mycobacterium</u> ^a sp.	2
	<u>Proteus</u> sp.	2
	<u>Pseudomonas</u> sp.	1
	<u>Pseudomona</u> <u>flurifescens</u> group	1
	<u>Salmonella</u> ^a sp.	1
3	<u>Clostridium perfringens</u>	1
	<u>Mycobacterium</u> sp.	50
	<u>Salmonella</u> ^a sp.	1
	<u>Mycobacterium</u> ^a sp.	13
	<u>Proteus</u> sp.	1
5	<u>Mycobacterium</u> ^a sp.	4
6	<u>Mycobacterium</u> ^a sp.	9
	<u>Proteus</u> sp.	3
Total		112

a = Class 2 Agent from Communicable Disease Center (1975),
Classification of Etiological Agents on the Basis of Hazard.

TABLE 82. MICROORGANISMS ISOLATED FROM COMMERCIAL LANDFILL
LEACHATE (PART II)

Leachate Sample	Microorganisms Identified	Number of Microbial Isolates Found
1	<u>Aspergillus niger</u>	1
	<u>Cephalosporium</u> sp.	1
	<u>Fusarium</u> sp.	1
	<u>Mycobacterium</u> ^a sp.	4
	<u>Neurospora</u> sp.	1
	<u>Proteus</u> sp.	9
	<u>Mycobacterium</u> ^a sp.	6
	<u>Proteus</u> sp.	4
	<u>Cephalosporium</u> sp.	1
	<u>Mycobacterium</u> ^a sp.	44
	<u>Proteus</u> sp.	2
	<u>Mycobacterium</u> ^a sp.	8
	<u>Penicillium</u> sp.	1
	<u>Proteus</u> sp.	2
	<u>Mycobacterium</u> ^a sp.	6
	<u>Neurospora</u> sp.	1
	<u>Proteus</u> sp.	3
	Total	94
	Combined Total	206

^a = Class 2 Agent, from Communicable Disease Center (1975,
Classification of Etiological Agents on the Basis of Hazard.

not on the CDC list, Clostridium perfringens is frequently involved in food poisoning and gas gangrene (Davis, et al., 1980). The other gram-negative rods are usually found in feces and in nature. The fungi listed are all found in nature.

b. Mycobacterium Levels

Table 83 shows the mycobacteria isolated from landfill leachate following concentration by diatomaceous earth filtration. To the far right of the table are the leachate volumes used in the concentration procedures. These volumes varied from one-half liter to one liter. Next, the mycobacterial levels found varied from 1 to 33/100 ml. Duplicate digestions processes were done during the first week to ascertain the precision of the procedure. The 4% NaOH was used in all samples while the 2% NaOH was used later in the last week for comparative purposes (Runyon et al., 1980).

The mycobacteria described in this table generally took 3 weeks to grow out. Isolates were later found to produce mature colonies within 6 days, indicating that they were rapid-growers. The colonies produced were of five types. Three were nonchromogenic; one was a smooth domed colony with a thin apron, a second was slightly rough with a serrated edge and a spot in the center and the third flat and wrinkled. The last two colony types were yellow and

TABLE 83. MYCOBACTERIA ISOLATED FROM THE COMMERCIAL LANDFILL
LEACHATE FOLLOWING CONCENTRATION BY FILTRATION^a

Sampling Date	Leachate Samples	Mycobacterium CFU/100 ml		Liters of Leachate Filtered
		NaOH Concentrations ^b		
		4%	2%	
06/09/80	1	2(<1) ^c	ND ^d	0.5
	2	<1(2)	ND	0.5
	3	32(20)	ND	0.5
06/16/80	4	13	ND	0.6
	5	4	ND	0.7
	6	9	ND	0.8
06/23/80	7	1	3	1.0
	8	1	5	1.0
	9	11	33	1.0
	10	1	7	1.0
	11	1	5	0.7

a = Diatomaceous earth

b = Used in the digestion process

c = Duplicate leachate digestion

d = Not determined

smooth: one was a photochromogen and the other was a scotochromogen (Lennette et al., 1974).

c. Grouping of Mycobacterium Isolates

Thus, the diatomaceous earth filter technique did concentrate a large number of mycobacteria from landfill leachate to allow for consistent isolations to be made. Some of the problems involved in isolations were due perhaps to dilution of microbes with excessive amounts of liquid. Thus, it becomes imperative to use large sample volumes in any assay.

To decide which of these isolates should be identified, we chose to group them using the drug susceptibility test. These results can be seen in Table 84. Down the left side of the table are listed the number of drug groups found, and across the top of the table are listed the drugs and their concentrations within the Middlebrook 7H10 agar. The negative symbol indicates that no growth occurred and that the bacteria were sensitive to the drug at that concentration. The plus symbol indicates that growth occurred and that the bacterium was resistant to that drug at the concentration listed. The thirteen drug groups indicate that at least thirteen strains were present. Their differences suggest that perhaps these mycobacteria did not have the same origin. The drug groups #7 and #8 contain the yellow mycobacteria strains, and are probably nonpathogenic,

TABLE 84. DRUG SUSCEPTIBILITY TESTS OF MYCOBACTERIA ISOLATED FROM LANDFILL LEACHATES
(PART I)

Drug Groups, ^a Identification Number	Number of Isolates per Group	Mycobacterium Growth in Agar ^b with mcg of Drug/ml										Control
		Ethambutol		Isoniazid		PAS ^c		Rifampin		Streptomycin		
		5	10	0.2	1	2	10	1	5	2	10	
1	2	- ^d	-	+ ^e	+	-	-	-	-	-	-	+
2	5	-	-	+	+	+	-	-	-	-	-	+
3	3	-	-	+	+	+	+	-	-	-	-	+
4	2	-	-	+	+	-	-	+	-	-	-	+
5	21	-	-	+	+	+	-	+	-	-	-	+
6	10	-	-	+	+	+	+	+	-	-	-	+
7	2	-	-	+	-	+	+	+	-	-	-	+
8	1	-	-	-	-	+	+	+	-	-	-	+
9 ^f	3	+	+	+	+	+	+	+	+	+	-	+
Subtotal	49											

a = Based on the drug susceptibility patterns.

b = Middlebrook 7H10 Agar.

c = P-aminosalicylic Acid

d = No growth.

e = Growth.

f = Mycobacteria taken from soil samples.

TABLE 84. DRUG SUSCEPTIBILITY TESTS OF MYCOBACTERIA ISOLATED FROM LANDFILL LEACHATES
(PART II)

Drug Groups, ^a Identification Number	Number of Isolates per Group	Mycobacterium Growth in Agar ^b with mcg of Drug/ml										Control
		Ethambutol		Isoniazid		PAS ^c		Rifampin		Streptomycin		
		5	10	0.2	1	2	10	1	5	2	10	
10	1	+	+	+	+	+	+	+	- ^d	+	-	+
11	2	-	-	+	+	-	-	+	-	+	-	+
12 ^e	1	+	-	+	+	+	+	+	+	+	-	+
13	1	-	-	+	+	+	-	-	-	+	-	+
<u>M. fortuitum</u> ATCC #6841	1	+	-	+	+	+	+	+	+	+	+	+
<u>M. goodii</u> ATCC #14470	1	-	-	+	-	-	-	-	-	-	-	+
<u>M. kansasii</u> ATCC #12478	1	-	-	+	-	-	-	+	+	+	+	+
Subtotal	8											
Subtotal from Part I	49											
TOTAL	57											

a = Based on the drug susceptibility patterns.

b = Middlebrook 7H10 Agar.

c = P-aminosalicylic Acid

d = No growth.

e = Growth.

f = Mycobacteria taken from soil samples.

while the other six groups are similar to the pathogenic nonchromogenic rapid-growing mycobacteria. Also, two groups came from the control soil.

In conclusion, although these strains isolated from the landfill leachates have not been completely identified, they do contain characteristics suggesting that both pathogenic and nonpathogenic mycobacteria may be present.

d. Significance of Pathogens Found in Leachate

The significance of finding the mycobacteria in landfill leachate was: (1) that they survived in the environment; (2) if they survived, then other more resistant mycobacteria, particularly Mycobacterium tuberculosis could probably also survive; (3) other microorganisms more resistant than the mycobacteria such as the sporeforming Clostridium would most certainly survive; (4) the survival of the high concentrations of the fecal indicators, the Salmonella, and Clostridium perfringens demonstrates the conditions under which the pathogens could survive in landfills. Their presence together with the Mycobacterium indicates that almost any microorganism of any sensitivity, equal to or greater than the Gram-negative rods found there such as the Salmonella and the fecal coliforms will survive landfill conditions.

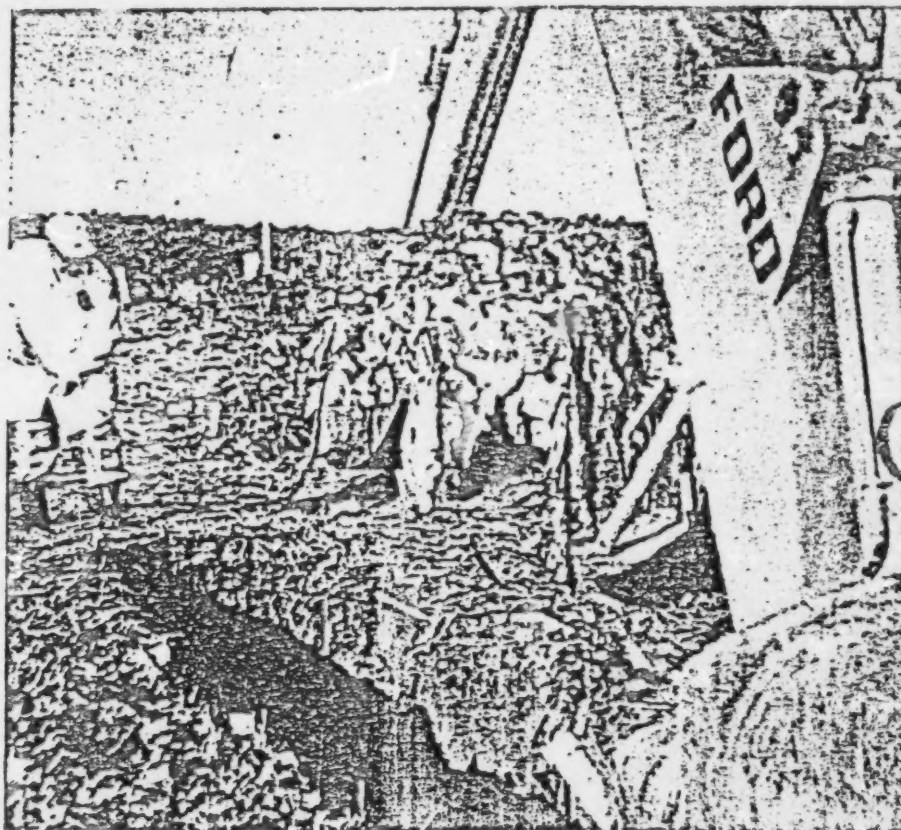
C. SOLID WASTE AND LEACHATE FROM A LANDFILL AT CLOSURE

1. General Information

The nine year old landfill site was examined in August, 1980. Two pictures of the site and one of the waste found in the bottom of the landfill are shown in Figure 21, 22, and 23. Figure 21 shows the hole dug in the landfill. Figure 22 shows the 10.5 ft. depth. At this point the temperature of the waste in August, 1980 was 14 C. Finally, Figure 23 shows the commercial sign placed in the landfill nine years ago. There is no decomposition. Finally, the solid waste and leachate taken from the landfill is described in Table 85. One explanation of the blue-green tint in the topsoil is that it was possibly due to a $\text{Fe}^{++} + \text{Fe}^{+++}$ complex in which the reduced form (the blue or the Fe^{++}) was present.

2. Fecal Indicators and Chemical Parameters

The fecal indicators levels were used as a measure of pollution, and these results are presented in Table 86 along with the conductivity, pH and moisture measurements in Table 87. The examination of the landfill solid wastes and leachate yielded unexpectedly high populations of indicator organisms. The small numbers of streptococci detected previously in the leachate became significant when the solid wastes from the landfill were examined after excavation. These results show that there were high levels of fecal



Reproduced from
best available copy.



FIGURE 21. THE SAMPLING SITE, 1980

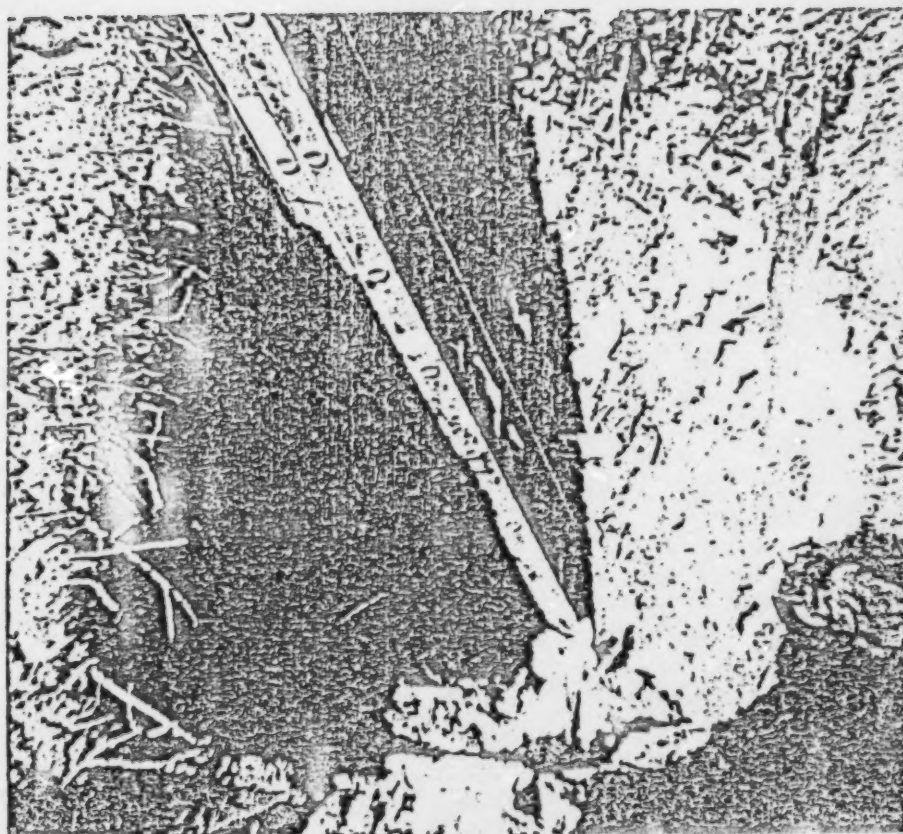
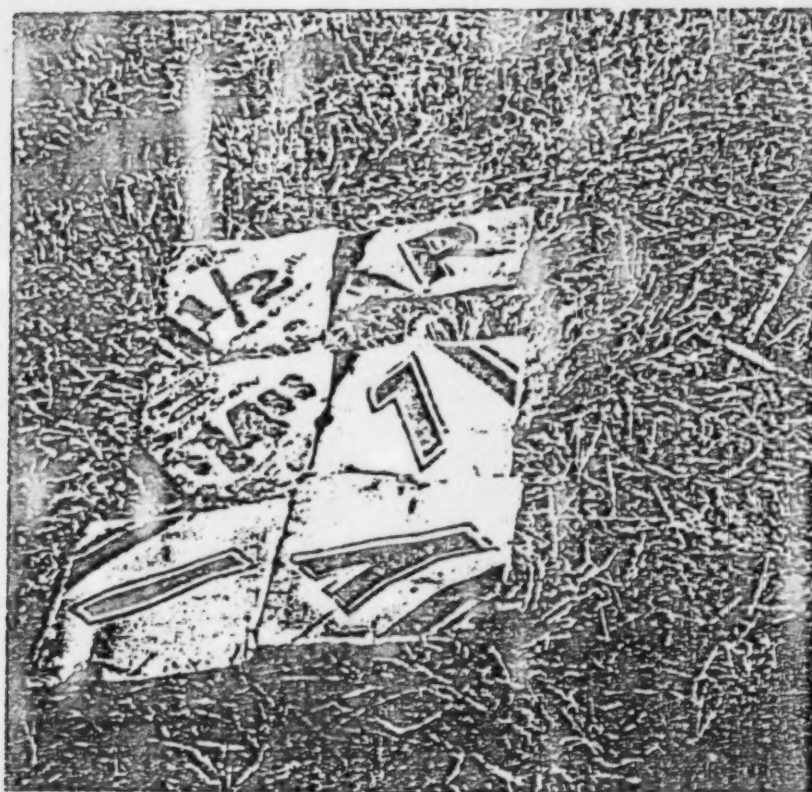


FIGURE 22. TC1 SITE WHEN A DEPTH OF 10 FEET WAS REACHED



Reproduced from
best available copy.



FIGURE 23. PAPER FROM EXHUMED TC1 WASTE SHOWING LACK OF DECOMPOSITION

TABLE 85. DESCRIPTION OF SAMPLES TAKEN FROM THE BOONE COUNTY LANDFILL

Samples	Samples Taken at These Descending Depths Ft (m)	Description of Sample
Top Soil Covering the Solid Waste	1.5 (0.46)	This soil was placed over the landfill. It was about 3 ft (0.9 m) in thickness. The clay soil just above the solid waste had a blue-green tint.
Solid Waste	5 (1.52)	This solid waste had settled and there was an irregular empty space between the topsoil and the solid waste below. The paper in the waste was not decomposed.
Solid Waste	7 (2.13)	The paper was not decomposed.
Solid Waste	8.5 (2.59)	The paper was not decomposed (see Figure 23).
Virus-Sample Probe Level	9 (2.74)	Viruses in containers with waste were added to this sample in 1971. Decomposed organic material was present.
Leachate	10 (3.05)	Leachate accumulated at this level in the landfill (see Figure 22).
Decomposed Material on Top of the Clay Liner	10.5 (3.20)	The decomposed material was black and slimy.
In Clay Liner	11 (3.35)	Clay soil had no apparent change from topsoil surface.
Under Clay and Plastic Liners	13 (3.96)	Clay soil had no apparent change from topsoil surface.

TABLE 86. FECAL INDICATOR BACTERIAL LEVELS IN SOLID WASTE AND LEACHATE FROM A NINE YEAR OLD LANDFILL^a

Type of Sample	Samples Collected at These Descending Levels		Fecal Indicator Bacteria MPN(CFU)/100g or 100 ml		
	Ft	(m)	Total Coliforms ^b	Fecal Coliforms ^b	Fecal Streptococci ^c
Top Soil	1.5	(0.46)	2.4X10 ⁵	3.5X10 ³	9.8X10 ⁵
MSW ^d	5.0	(1.52)	1.1X10 ²	<2.0X10 ¹	2.0X10 ⁴
MSW	7.0	(2.13)	2.4X10 ³	2.0X10 ¹	6.0X10 ⁴
MSW	8.5	(2.60)	1.6X10 ⁵	2.3X10 ²	3.3X10 ⁵
MSW	9.0	(2.74)	5.4X10 ⁴	3.3X10 ²	ND ^e
Leachate	10.0	(3.01)	9.2X10 ³	3.5X10 ²	1.1X10 ²
MSW	10.5	(3.20)	3.5X10 ⁴	4.9X10 ²	3.5X10 ⁴
Clay Liner	11.0	(3.35)	5.0X10 ¹	<2.0X10 ¹	2.0X10 ⁴
Soil Beneath	13.0	(3.96)	5.0X10 ¹	<2.0X10 ¹	<2.0X10 ⁴

a = Boone County field site, Test Cell 1.

b = Standard Methods (1976) adapted by Allen et al., (1976).

c = Standard Methods (1976) plus the use of the Gram stain to check the fecal positive tests for cocci.

d = Municipal Solid Waste

e = Not Determined.

TABLE 87. BOONE COUNTY LANDFILL FIELD SITE, TEST CELL 1, PHYSICAL-CHEMICAL CHARACTERISTICS

Type of Sample	Samples Collected at These Descending Levels		pH	Conductivity umhos/cm	Moisture %
	Ft	(m)			
Top Soil	1.5	(0.46)	7.6	200	16.3
MSW ^a	5.0	(1.52)	6.0	1,890	63.5
MSW	7.0	(2.13)	5.4	1,330	73.0
MSW	8.5	(2.60)	7.4	1,930	51.5
MSW	9.0	(2.74)	7.6	ND ^b	80.0
Leachate	10.0	(3.01)	7.0	1,399	ND
MSW ^c	10.5	(3.20)	6.9	ND	63.6
Clay ^d	11.0	(3.35)	6.3	ND	ND
Soil ^e	13.0	(3.96)	6.2	ND	20.5

a = Municipal Solid Waste

b = Not Determined

c = Decomposed Waste at Plastic Liner

d = Clay Liner

e = Soil Under Clay Liner

indicator levels in the 1-1/2 ft soil sample . Following this there were lower fecal indicator levels at the five foot sample and then higher levels for the rest of the wastes down to the bottom of the landfill.

The total coliforms, fecal coliform, and the fecal streptococci levels of all waste material were high and significant. For example, a level of greater than 10^2 fecal coliform/100 ml of water, may be considered significant and therefore polluted (Geldreich, 1976). In addition to the high total coliform and fecal coliform levels, the fecal streptococci varied from only 10^4 to 10^5 /100 g in the solid waste. The low level of streptococci in the leachate was inconsistent with the other fecal indicator sample concentrations. However, it would appear that the streptococci were the more stable landfill microorganism when compared to the coliforms as they maintained higher levels. On the other hand, the topsoil fecal indicator levels had higher levels compared with the wastes taken at lower depths. This was perhaps due to recent pollution or maybe the pollution placed there nine years ago. The higher fecal indicator levels at the top indicated that the fecal material was washed down from the surface and it drained down through the landfill. Or perhaps these fecal indicator bacteria could be the remains of the original waste placed there nine years ago.

At greater depths the counts decreased and then increased again (Table 87). There is no ready explanation for this finding. Also, going below the waste, the results of the clay liner and the soil beneath the plastic liner showed that few bacterial indicators were present. This assay demonstrated that the microbial-containing leachate was not able to penetrate the clay liner and contaminate the soil and groundwater beneath.

Table 87 gives some of the physical-chemical characteristics found. The topsoil pH was high, but the following two levels were low. This low pH may have been responsible for the decreased microbial counts at these levels. Also, the temperature in the summertime was high in these upper levels, and fermentation was able to be carried out by the coliforms. Such activity would produce acids and carbon dioxide, which could bring about an increased acidic condition, and lower the bacterial counts. At lower levels, 10.5 ft (3.2 m) below the ground surface, the temperature of the solid waste was 14 C. The lack of conductivity in the topsoil indicated that little mineralization had occurred and this would explain the high pH of 7.6, compared to that of the leachate (7.0). This high pH appeared to be favorable because the microbial counts remained high and did not decrease with time as occurred in the other levels. The percent moisture was low in the soil and high in the solid

waste. Thus, the lack of moisture in the soil along with the low conductivity, high pH, and high microbial levels all suggest that the microorganisms just stayed in the soil and did not metabolize. On the other hand, the microorganisms in the solid waste mineralized some of the waste material, and these microbes may have had enough moisture to survive, metabolize, and multiply. The solid waste was very moist. These fecal indicators could thus remain for long periods of time and contaminate the environment when uncovered.

A comparison of the landfill samples with the control soils (see Table 88 and 89) demonstrated significantly different populations in the solid waste compared to the soil control. The total coliform and the fecal streptococci levels were high, but the fecal coliforms were not present in the soil control, while at the lower depths there were no fecal indicators at all. The control soil was acidic on top, had low conductivity and moisture levels. All characteristics were different from those in the landfill. Thus, one must conclude that the high fecal indicator levels were derived from the solid waste placed in this site nine years ago.

3. Plate Counts

A comparison of Table 90 and '91, which give the plate count levels of the landfill soil and solid waste at descending levels, reveals the presence of higher levels of

TABLE 88. FECAL INDICATOR BACTERIAL LEVELS IN THE SOIL CONTROL SAMPLES

Type of Sample	Samples Collected at These Descending Levels		Fecal Indicator Bacteria MPN(CFU)/100g or 100 ml			
	Ft	(m)	Total Coliform ^a	Fecal Coliform ^a	Fecal Streptococci ^b	
Top Soil	1.5	(0.46)	>2.4X10 ⁵	< 20	2.1X10 ³	
Soil	5.0	(1.52)	2.3X10 ²	< 20	2.3X10 ²	
Soil	7.0	(2.13)	50	< 20	< 20	
Soil	8.5	(2.59)	50	< 20	50	

a = Standard Methods (1976) adapted by Allen et al., (1976).

b = Standard Methods (1976) modified by the addition of the Gram stain to the final step for the microscopic examination of cocci.

TABLE 89. CONTROL SOIL NEAR BOONE COUNTY LANDFILL, PHYSICAL-CHEMICAL CHARACTERISTICS AT DESCENDING LEVELS

Type of Sample	Samples Collected at These Descending Levels		pH	Conductivity umhos/cm	Moisture %
	Ft	(m)			
Top Soil	1.5	(0.46)	5.7	56	17.3
Soil	5.0	(1.52)	5.4	72	18.5
Soil	7.0	(2.13)	7.0	38	24.1
Soil	8.5	(2.59)	7.7	38	20.8

TABLE 90. MICROBIAL CONCENTRATIONS IN SOIL, SOLID WASTE AND LEACHATE FROM THE BOONE COUNTY FIELD SITE TC-1 ON 8/22/80 (PART I)

Quantitative Tests	Microbial Concentrations of Landfill Contents at Descending Levels			
	Topsoil at 1-1/2 ft (0.46m)	Solid Waste 5 ft (at 1.52m)	Solid Waste 7 ft (at 2.13m)	Solid Waste 8-1/2 ft (at 2.60m)
<u>Fecal Indicator Levels</u> MPN/100 g				
Total Coliform ^a	2.4X10 ⁵	1.1X10 ²	2.4X10 ³	1.6X10 ⁵
Fecal Coliform ^a	3.5X10 ³	< 20	2.0X10 ¹	2.3X10 ²
Fecal Streptococci ^b	9.8X10 ⁵	2.0X10 ⁴	6.0X10 ⁴	3.3X10 ⁵
<u>Agar Plate Counts</u> CFU/100g				
Standard Methods	2.7X10 ⁹	3.2X10 ⁶	2.5X10 ⁷	3.6X10 ⁷
Blood-Aerobic	4.0X10 ⁸	6.3X10 ⁶	3.0X10 ⁷	1.1X10 ⁸
Blood-Anaerobic	3.5X10 ⁸	1.0X10 ⁷	6.0X10 ⁶	1.0X10 ⁸
KF Streptococcal	1.0X10 ²	1.0X10 ⁴	1.0X10 ⁴	1.0X10 ⁴
MacConkey	4.3X10 ⁶	2.0X10 ⁴	7.0X10 ⁴	3.6X10 ⁵
Inhibitory Mold	4.8X10 ⁶	5.0X10 ⁴	5.0X10 ⁴	9.0X10 ⁴
Sabouraud	1.5X10 ⁷	1.0X10 ⁴	1.0X10 ⁴	1.0X10 ⁴
g-Enterococcus	9.8X10 ⁴	2.0X10 ³	6.0X10 ⁴	3.3X10 ⁵

a = Standard Methods (1976) as modified by Allen et al., (1976).

b = Standard Methods (1976) as modified by the adding of the Gram stain to the final step for the microscopic examination of cocci.

c = Not determined.

TABLE 90. MICROBIAL CONCENTRATIONS IN SOIL, SOLID WASTE AND LEACHATE FROM THE BOONE COUNTY FIELD SITE TC-1 ON 8/22/80 (PART II)

Quantitative Tests	Microbial Concentrations of Landfill Contents at Descending Levels		
	Leachate	Clay Liner	Beneath Liner
	10 ft (3.01m)	11 ft (1.52m)	13 ft (3.96m)
<u>Fecal Indicator Levels</u> MPN/100 g (ml)			
Total Coliform ^a	9.2X10 ³	2.0X10 ²	7.9X10 ²
Fecal Coliform ^a	3.5X10 ²	8.0X10 ¹	2.0X10 ¹
Fecal Streptococci ^b	1.0X10 ²	ND ^c	ND
<u>Agar Plate Counts</u> CFU/100g			
Standard Methods	3.0X10 ⁷	9.0X10 ⁶	2.5X10 ⁷
Blood-Aerobic	7.5X10 ⁷	1.0X10 ⁷	1.2X10 ⁷
Blood-Anaerobic	2.0X10 ⁶	2.0X10 ⁵	1.0X10 ⁴
KP Streptococcal	1.0X10 ⁴	1.0X10 ⁴	1.0X10 ⁴
MacConkey	6.3X10 ⁵	1.0X10 ⁵	6.0X10 ⁴
Inhibitory Mold	4.0X10 ⁴	1.0X10 ⁴	1.0X10 ⁴
Sabouraud	1.0X10 ⁴	1.0X10 ⁴	1.0X10 ⁴
m-Enterococcus	ND	2.0X10 ³	2.0X10 ³

a = Standard Methods (1976) as modified by Allen et al., (1976).

b = Standard Methods (1976) as modified by the adding of the Gram stain to the final step for the microscopic examination of cocci.

c = Not determined.

TABLE 90. MICROBIAL CONCENTRATIONS IN SOIL, SOLID WASTE AND LEACHATE FROM THE
BOONE COUNTY FIELD SITE TC-1 ON 8/22/80 (PART III)

Quantitative Tests	Microbial Concentrations of Landfill Contents at Descending Levels			
	Sample Probe	Top of Plastic	Clay Liner	Beneath Liner
	9 ft (2.74m)	10-1/2 ft (3.20m)	11 ft (1.52m)	13 ft (3.96m)
<u>Fecal Indicator Levels</u> MPN/100 g				
Total Coliform ^a	5.4X10 ⁴	3.5X10 ⁴	5.0X10 ¹	5.0X10 ¹
Fecal Coliform ^a	3.3X10 ²	4.9X10 ²	< 20	< 20
Fecal Streptococci ^b	ND ^c	3.5X10 ⁴	ND	ND
<u>Agar Plate Counts</u> CFU/100g				
<u>Standard Methods</u>	ND	4.8X10 ⁷	1.0X10 ⁷	9.0X10 ⁶
Blood-Aerobic	1.8X10 ⁸	7.7X10 ⁷	1.7X10 ⁷	1.8X10 ⁶
Blood-Anaerobic	3.0X10 ⁷	8.4X10 ⁵	6.0X10 ⁶	1.0X10 ⁶
KP Streptococcal	ND	ND	ND	ND
MacConkey	8.7X10 ⁸	8.5X10 ⁵	1.0X10 ⁴	1.0X10 ⁴
Inhibitory Mold	1.0X10 ⁴	2.0X10 ⁴	7.0X10 ⁴	6.0X10 ⁴
Sabouraud	1.0X10 ⁴	5.0X10 ⁴	1.2X10 ⁶	2.9X10 ⁵

a = Standard Methods (1976) as modified by Allen et al., (1976).

b = Standard Methods (1976) as modified by the adding of the Gram stain to the final step for the microscopic examination of cocci.

c = Not determined.

TABLE 91. MICROBIAL CONCENTRATIONS OF SOIL CONTROL NEAR THE BOONE COUNTY FIELD SITE TC-1 ON 9/9/80

Quantitative Tests	Microbial Concentrations of Landfill Contents at Descending Levels			
	Topsoil at 0.46m (1-1/2 ft)	Soil at 1.52m (5 ft)	Soil at 2.13m (7 ft)	Soil at 2.60m (8-1/2 ft)
Fecal Indicator Levels				
MPN/100 g				
Total Coliform ^a	2.4X10 ⁵	2.3X10 ²	5.0X10 ¹	5.0X10 ¹
Fecal Coliform ^a	< 20	< 20	< 20	< 20
Fecal Streptococci ^b	2.1X10 ³	2.3X10 ²	< 20	5.0X10 ¹
Agar Plate Counts				
CFU/100g				
<u>Standard Methods</u>	6.9X10 ⁸	2.9X10 ⁶	3.8X10 ⁴	5.9X10 ⁴
Blood-Aerobic	7.2X10 ⁸	1.6X10 ⁶	1.4X10 ⁵	3.0X10 ⁵
Blood-Anaerobic	4.8X10 ⁸	2.0X10 ⁵	1.0X10 ⁵	1.0X10 ⁵
KF Streptococcal	ND ^c	ND	ND	ND
MacConkey	4.5X10 ⁷	4.0X10 ⁴	1.0X10 ⁴	2.0X10 ⁴
Inhibitory Mold	6.7X10 ⁶	3.0X10 ⁴	1.0X10 ⁴	1.0X10 ⁴
Sabouraud	1.0X10 ⁴	2.0X10 ⁵	1.0X10 ⁴	1.1X10 ⁵
m-Enterococcus	2.0X10 ³	2.0X10 ³	2.0X10 ³	2.0X10 ³

a = Standard Methods (1976) as modified by Allen et al., (1976).

b = Standard Methods (1976) as modified by the adding of the Gram stain to the final step for the microscopic examination of cocci.

c = Not determined.

bacteria in the landfill than that of the soil controls, i.e., the total coliform, fecal coliforms, and the fecal streptococci were higher, and the plate counts were somewhat higher in selected instances. These recovered bacteria were able to exist in either laboratory lysimeters or in the full-scale landfill for years. The significant changes in this study involved the decrease in the concentration of the coliforms and the streptococci.

Aerobic and anaerobic blood agar plates were used to enumerate the Clostridium perfringens that might be present. No large zones of hemolysis were found on the anaerobic blood plate which indicated the presence of C. perfringens. When present, C. perfringens would grow out within 24 or 48 hours. This incubation period was probably too short to pick up other forms of anaerobes. The C. perfringens methods described in Chapter 4 were not available for this experiment.

4. The Identification of Gram-Negative Rods

The identification of the Gram-negative bacteria isolated from the several levels of the landfill are presented in Table 92 and 93. A survey of the microorganisms found in the top soil and waste indicate that these are soil bacteria plus fecal bacteria including a few pathogens such as Salmonella, Arizona, Klebsiella pneumoniae, Moraxella, and Acinetobacter. The top soil bacteria appear to be soil

TABLE 92. GRAM-NEGATIVE RODS ISOLATED^a FROM THE BOONE COUNTY LANDFILL TC-1 AND THE CONTROL SOIL (PART I)

Sample Source	MacConkey Plate Counts CFU/100 g	Isolated Microorganisms Name
Topsoil (0.46 m) 1-1/2 ft	4.3X10 ⁴	<u>Achromobacter</u> sp. <u>Enterobacter agglomerans</u> <u>Flavobacterium</u> sp. <u>Moraxella</u> ^b sp. <u>Pseudomonas</u> sp.
Solid Waste (1.52 m) 5 ft	2X10 ²	None Isolated
Solid Waste (2.13 m) 7 ft	7X10 ²	<u>Acinetobacter</u> sp. ^b <u>Proteus mirabilis</u> <u>Serratia marcescens</u>
Solid Waste (2.60 m) 8-1/2 ft	3.6X10 ³	<u>Enterobacter aerogenes</u> <u>Hafnia</u> sp. <u>Klebsiella pneumoniae</u> ^b <u>Pseudomonas</u> sp.

a = Isolated from MacConkey Agar Plates.

b = Class 2 Agents, Communicable Disease Center (1974).

TABLE 92. GRAM-NEGATIVE RODS ISOLATED^a FROM THE BOONE COUNTY LANDFILL TC-1 AND THE CONTROL SOIL (PART II)-CONTINUED

Sample Source	MacConkey Plate Counts CFU/100 g	Isolated Microorganisms Name
Virus Sample Probe Level (2.74 m) 9 ft	8.7X10 ⁵	<u>Arizona</u> sp. ^b <u>Serratia</u> sp.
Leachate (3.01 m) 10 ft	6.3X10 ³	<u>Citrobacter diversans</u> <u>Enterobacter aerogenes</u> <u>Enterobacter agglomerans</u> <u>Enterobacter cloacae</u> <u>Enterobacter sakazaki</u> <u>Hafnia</u> sp. <u>Proteus</u> sp. <u>Providencia</u> sp. <u>Serratia marcescens</u>
Top of Plastic Liner (3.20 m) 10-1/2 ft	8.4X10 ³	<u>Enterobacter agglomerans</u> <u>Enterobacter</u> sp. <u>Proteus</u> sp. <u>Serratia</u> sp.

a = Isolated from MacConkey Agar Plates.

b = Class 2 Agents, Communicable Disease Center (1974).

TABLE 92. GRAM-NEGATIVE RODS ISOLATED^a FROM THE BOONE COUNTY LANDFILL TC-1 AND THE CONTROL SOIL (PART III)

Sample Source	MacConkey Plate Counts CFU/100 g	Isolated Microorganisms Name
Clay Liner (3.35 m) 11 ft	1X10 ²	<u>Enterobacter aerogenes</u> <u>Enterobacter agglomerans</u> <u>Enterobacter sp.</u> <u>Klebsiella sp.b,c</u> <u>Proteus sp.</u> <u>Serratia sp.</u>
Under Clay Liner (3.96 m) 13 ft	10 ²	None ^d
<u>Control Soil</u>		
Top Soil (0.46 m) 1-1/2 ft	4.4X10 ⁵	Total Coliforms ^b
Soil (1.52 m) 5 ft	4X10 ²	None
Soil (2.13 m) 7 ft	1X10 ²	None
Soil (2.60 m) 8-1/2 ft	2X10 ²	None

a = Isolated from MacConkey Agar Plates

b = Presumptive identification

c = Class 2 Agent, Communicable Disease Center (1974)

d = No Enterobacteriaceae were isolated

TABLE 93. MICROORGANISMS ISOLATED ON HEKTOEN ENTERIC AGAR
FROM BOONE COUNTY LANDFILL, TC-1

Sample Source	Identified Microorganisms	Presence ^a of <u>Salmonella</u>
<u>Landfill Samples</u>		
Top Soil	<u>Citrobacter</u> sp.	-
1-1/2 ft (0.46 m)	<u>Escherichia coli</u>	
Solid Waste	<u>Enterobacter cloacae</u>	
5 ft (1.52 m)	<u>Enterobacter agglomerans</u>	+
	<u>Escherichia coli</u>	
Solid Waste	<u>Hafnia</u> sp.	-
7 ft (2.13 m)	<u>Serratia liquefaciens</u>	
Solid Waste	<u>Escherichia coli</u>	-
(2.60 m) 8-1/2 ft		
Leachate	<u>Enterobacter aerogenes</u>	-
(3.01 m)		
10 ft	<u>Proteus</u> sp.	
Clay Liner	<u>Escherichia cloacea</u>	-
(3.35 m)		
11 ft	<u>Escherichia coli</u>	
Under Clay Liner	<u>Escherichia coli</u>	-
(3.96 m) 13 ft		
<u>Control Soil</u>		
Soil at 11/2, 5, 7 and 8-1/2 ft (0.46, 1.52, 2.13, and 2.60 m	None	-

a = Solid Waste and soil samples were placed in brain heart infusion broth for two hours and then placed in Selenite F broth and into GN broth enrichment broths. These media were incubated for 24 to 48 hours at 37 C and streaked on Hektoen enteric agar for isolation purposes. The Salmonella is a Class 2 Agent, Communicable Disease Center (1974).

Gram-negative rods and are consistent with those usually found in soil. The rest of the bacteria appear to be fecal bacteria. Such results are consistent with those usually found in soil and are consistent with the MPN fecal-indicator tests.

In Table 93 the results of the enrichment tests for Salmonella are presented along with the microorganisms isolated from the Hektoen enteric plates, which were later identified. Although most microbes did not turn out to be Salmonella sp., they were recognized as fecal bacteria. These same bacteria were not found in the soil control. The one Salmonella identified along with the presence of the many fecal Gram-negative rods was significant. Infections can be caused by the microorganisms identified in the Boone County landfill (TC-1) of Walton, Kentucky.

The human pathogens have been identified in Tables 92 and 93. These bacteria are Class 2 agents (Communicable Disease Center, 1974) and can, under the right conditions, produce human infections. Their presence in the waste after nine years suggests that other similar bacteria may also be present.

The source of these pathogens has not been determined but could be (1) animal feces deposited on the surface of the landfill. Animals are known to forage on deposited waste or landfills (E.E. Geldreich, 1982 ; personal

communication). Their feces may remain in the topsoil for undetermined periods of time and may be washed down through the buried waste. Also (2) municipal waste could be the source. Under optimum conditions of temperature (14 C), pH, and moisture the microorganisms may last for indefinite periods of time in the depths of the landfill.

D. CONCLUSIONS

Samples from the nine year old Boone County landfill TC-1, and soil controls, were examined microbiologically. Significant levels of fecal indicators, (i.e., total and fecal coliforms and fecal streptococci) were also found in the landfill solid wastes at all depths, indicating that these microbes had continued to survive and possibly reproduce for nine years. The low or non-existent microbial presence in the leachate samples draining out of the landfill over the past nine years did not, therefore, indicate the true microbial numbers residing in the solid wastes contained within the landfill itself. The high microbial numbers found in the leachate obtained within the landfill, and the high fecal-indicator levels present in the land-filled solid wastes, demonstrated that there was recent animal contamination or that microbes can continue to dwell within the landfill for indefinite lengths of time, in this case nine years. Landfills must therefore be considered as containing high numbers of fecal bacteria, instead of

disregarding the microbial population of these waste disposal facilities as is the present policy. In addition, the identified pathogens found in the solid waste samples from TC-1 included Acinetobacter sp., Moraxella sp., Salmonella sp., and Klebsiella pneumoniae. Other nonpathogenic Enterobacteriaceae and related Gram-negative rod bacteria were also found. These microorganisms were not found in the control soils. Therefore, the presence of these microorganisms in the solid waste of landfill is thus significant.

CHAPTER VIII

CONCLUSIONS

1. Methods were modified by applying bacterial stress procedures to bring about the enumeration and isolation of coliforms, streptococcus, Mycobacterium, Salmonella, and Clostridium.

2. Leachates and solid waste obtained both from an active and inactive (9 yr) landfill contained fecal coliform levels of 10^3 MPN/100 ml and fecal streptococci of 10^5 MPN/100 ml. Also, the following pathogens were identified from the leachate: Clostridium perfringens, Salmonella sp., Klebsiella sp., Listeria sp., Proteus sp., Providencia sp., Mycobacterium sp., Moraxella sp., Acinetobacter sp., and Streptococcus faecalis, suggesting that fecal matter and other animal or human wastes were present in the landfill. Some investigators believe that the pathogens from the older landfills were derived when wild animals defecated on the surface and not from the waste originally deposited there. This may be true, but regardless of the origin, pathogens were found in solid waste or leachate derived from the landfill. Therefore, this work suggested that the leachate could contaminate areas where it drained, and that the solid waste material in the landfill might serve as a continuing source of both pathogens and fecal indicator microbes.

3. Over the years, fecal indicator bacteria present in leachate samples decreased in numbers, often to below detectable levels and occasionally up to 10^2 MPN/100 ml in inactive landfills, such as the Boone County, Kentucky, landfill. These results suggested that the bacteria had all but died out. However, this was not the case, as noted in (1) above.

4. The commercial landfill leachate contained species of both oral and fecal streptococci. Streptococcus faecalis was the only streptococcus found in older landfills and lysimeters. That this species is found mainly associated with man and animals indicates the origin of the waste, and suggested that other microorganisms, both pathogenic and nonpathogenic, also of a similar origin and resistance will also be present.

5. Newly constructed experimental lysimeters at the University of Cincinnati, containing municipal solid waste, hospital waste, and sewage sludge, the following conclusions were made:

- a. Fecal coliform numbers decreased below detectable levels in the leachate after four months while the fecal streptococci levels were present for a year. Thus, in the six experimental lysimeters streptococci survived for shorter periods of time (1 year vs. 7 years) than those in the

Boone County, Kentucky, landfill, suggesting that all of the pathogens had died out in the landfills. Again, by examining the solid waste itself within the landfill, we found that this was not the actual case.

- b. The fecal indicator levels (i.e. fecal streptococci) found in both hospital wastes and sewage sludge mixed with municipal wastes were consistently higher for longer time periods than those found in municipal waste or hospital waste alone over the same time. Perhaps the mixed waste offered the better bacterial refuge.
- c. Sewage sludge bacteria died within the sewage sludge mass but retained a long viability on the surface of the sludge probably because of their exposure to weekly water additions and the subsequent removal of toxic wastes.
- d. The levels of Clostridium perfringens in the leachate (ranging from 1 to 10^2 MPN/100 ml) and the high levels in the solid waste (ranging from 10^5 to 10^7 MPN/100 g) suggested that these bacteria survived for years in landfills.
- e. The presumptive identification of C. botulinum and C. tetani, in addition to C. perfringens, suggests that the leachate may contain toxins

produced by these microorganisms. Drinking or bathing in the leachate contaminated water could be hazardous as the toxins may cause food poisoning or tetanus.

CHAPTER IX

RECOMMENDATIONS

1. Leachate leaving landfills should be considered as sewage or diluted sludge and treated accordingly because of the presence of sewage-type microorganisms. With time, landfill bacteria decrease in numbers, but levels of 10^3 to 10^5 fecal coliforms and fecal streptococci MPN, could remain for considerable periods of time, measurable in years.

2. In a field study workers should monitor wells and surface waters near landfills for fecal indicators, pathogens (including mycobacteria) and clostridial toxins to determine the effect of landfills on the drinking water supply.

3. For the purpose of microbial monitoring of landfill sites, a set of procedures should be developed that can be used all over the United States to determine the fecal indicator and the pathogenic bacterial levels. The monitoring methods should include fecal indicator tests with microbial stress-reducing procedures, bacterial concentrations procedures for pathogens, quantitative tests for enteric pathogens including Mycobacteria tuberculosis, Legionella sp., the enteric viruses, and the clostridial toxins.

4. Finally, the adverse effects on humans and animals after drinking leachate-contaminated water or water contaminated with anaerobically digested material should be determined. This information would aid both the population and the landfill operators to decide which land should be used for the landfill site and for deciding the optimum distance between landfills and residential areas using wells for drinking purposes.

BIBLIOGRAPHY

- Adams, D. M. 1974. Requirement for and sensitivity to lysozyme by Clostridium perfringens spores heated at ultra-high temperatures. Appl. Microbiol. 27:797-801.
- Albritton, W.L., G.L. Wiggins, W.E. DeWitt, and J.C. Feeley, 1980. Listeria monocytogenes, pp. 139-142. In E.H. Lennette, A. Balows, W.J. Hausler, Jr., and J.P. Truant, (eds.) Manual of Clinical Microbiology, 3rd Ed. American Society for Microbiology, Washington, D.C.
- Alexander, M. 1977. Introduction to Soil Microbiology, 2nd ed. John Wiley & Sons, Inc., New York. pp. 154-159; 233-235.
- Allen, M. J., R. H. Taylor, and E. E. Geldreich. 1976. The impact of excessive bacterial population on coliform methodology. Am. Water Works Assoc. Water Quality Technology Conference, San Diego, Calif., Dec. 6 and 7, 1976.
- American Public Health Association. 1976. Standard methods for the examination of water and wastewater, 14th ed., American Public Health Association, New York.
- Analytab Products. 1977. Analytical Profile Index, Enterobacteriaceae and other Gram negative bacteria. Analytab Products, Plainview, New York.
- Analytab Products. 1979. Analytical Profile Index, Enterobacteriaceae and other Gram negative bacteria. Analytab Products, Plainview, New York.
- Analytab Products. 1978. Analytical Profile Index, anaerobes. Analytab Products, Plainview, New York.
- Angelotti, R., H.E. Hall, M.J. Foter, and K.H. Lewis. 1962. Quantitation of Clostridium perfringens in Foods. Applied Microbiology 10:193-199.
- Bacteriological examination of water supplies, Report on Public Health and Medical Subjects, #71. Dept. of Health and Social Security, Dept. of Environment, Her Majesty's Stationary Office, London, pp. 7-8, 32-35.

- Bailey, W.R. and E.G. Scott. 1974. Diagnostic microbiology, a textbook for the isolation and identification of pathogenic microorganisms, 4th ed. The C.V. Mosby Co., St. Louis.
- Barach, J.T., R.S. Flowers, and D.M. Adams. 1975. Repair of heat-injured Clostridium perfringens spores during outgrowth. Appl. Microbiol. 30:873-875.
- Beneke, E.S. and A.L. Rogers. 1970. Medical Mycology Manual. 3rd Ed. Burgess Publishing Co., Minneapolis, Minn.
- Berg, G. 1974. The Virus Hazard - A Panorama of the Past, A Presage of Things to Come. p. xiii-xvii. In J. F. Milina, Jr. and B. P. Sagik (eds.) Virus Survival in Water and Wastewater Systems, Water Resources Symposium Number Seven. Center in Water Resources, University of Texas, Austin.
- Berk, W.J. and B.S. Yare. 1977. An Integrated Approach to Delineating Contaminated Ground Water. Ground Water 15:138-145.
- Beuchat, L.R., and R.V. Lechowich. 1968. Effect of salt concentration in the recovery medium on heat-injured Streptococcus faecalis, Appl. Microbiol 16:772-776.
- Bissonnette, G.K., J.J. Jazeski, G.A. McFeters, and D.G. Stuart. 1975. Influence of environmental stress on enumeration of indicator bacteria from natural waters. Appl. Microbiol. 29:186-194.
- Bissonnette, G.K., J.J. Jazeski, G.A. McFeters, and D.G. Stuart. 1977. Evaluation of recovery methods to detect coliforms in water. Appl. Environ. Microbiol. 33:590-595.
- Blannon, J.C. and M.L. Peterson. 1974. Survival of fecal coliforms and fecal streptococci in a sanitary landfill. News of Environmental Research in Cincinnati, April 12, 1974. U. S. Environmental Protection Agency, Cincinnati.
- Block, S.S., and J.C. Netherton. 1977. Infectious hospital wastes, their treatment and sanitary disposal, pp.723-739. In S. S. Block (ed.) Disinfection, sterilization and preservation. 2nd ed. Lea and Febiger, Philadelphia.

- Bonde, G. 1963. Bacterial indicators of water pollution. A study of quantitative estimation. In Tekniskforlag, 2nd ed. Copenhagen.
- Bonde, G.J. 1974. Bacterial indicators of sewage pollution. International Symposium on Discharge of Sewage from Sea Outfalls, London. 23 August, Her Majesty's Stationary Office.
- Bordner, R., J. Winter, and P.V. Scarpino, (eds). 1978. Microbiological methods for monitoring the environment, water and wastes, Environmental Monitoring and Support Laboratory, Environmental Protection Agency, Cincinnati, Ohio, EPA-600/8-78-017.
- Branson, D. 1972. Methods in clinical bacteriology, a manual of tests and procedures. Charles C. Thomas, Springfield, Illinois.
- Braswell, J.R., and A.W. Hoadley. 1974. Recovery of Escherichia coli from chlorinated secondary sewage. Appl. Microbiol. 28:328-329.
- Brewer, D.G., S.E. Martin, Z.J. Ordal. 1977. Beneficial effect of catalase or pyruvate in a most-probable-number technique for the detection of Staphylococcus aureus. Appl. Microbiol. 34:797-800.
- Brown, L.R., R.J. Strawinski and C.S. McCleskey. 1964. The isolation and characterization of Methanonas methanoxidans, Brown and Strawinski. Canad. J. Microbiol., 10:791-799.
- Brown, R.H. 1964. Hydrologic Factors Pertinent Groundwater Contamination. Ground Water 2:5-12.
- Brunner, D.R., and D.J. Keller. 1972. Sanitary Landfill Design and Operation. Report SW-65ts. United States Environmental Protection Agency, Cincinnati, Ohio.
- Buchanan, R.E., N.E. Gibbons, S.T. Cowan, J.G. Holt, J. Liston, R.G.E. Murray, C.F. Niven, A.W. Ravin, and R.Y. Stanier. 1974. Bergy's manual of determinative bacteriology, 8th ed. The Williams and Wilkins Co., Baltimore.
- Burge, W.D. and P.B. Marsh. 1978. Infectious disease hazards of land spreading sewage waters. J. Env. Quality. 7:1-6.

- Burchinal, J.C. and L.P. Wallace. 1971. A study of institutional solid wastes. Department of Civil Engineering, West Virginia University, Morgantown.
- Butler, R.G., G.T. Oblob, and P.H. McGauhey. 1954. Underground Movement of Bacterial and Chemical Pollutants. J. Amer. Water Works Assoc. 46:97-111.
- Cabelli, V.J. 1976. Clostridium perfringens as a water quality indicator, pp.65-79. In A. W. Hoadley and B. J. Dutka (eds.) Bacterial indicators/health hazards associated with water. A Symposium sponsored by ASTM, Chicago, Ill., June 28-29, 1976, American Society for Testing and Materials, Philadelphia, Pa.
- Cameron, R.D., and E.C. McDonald. 1977. Coliforms and municipal landfill leachate. J. Water Poll. Control Fed. 49:2504-2506.
- Carpenter, L.V., and L.R. Setter. 1940. Some notes on sanitary landfills. Amer. J. Pub. Health 30:385-393.
- Carter, G.R. 1979. Diagnostic procedures in veterinary bacteriology and mycology. 3rd ed. Chas C. Thomas, Publisher. Springfield, Illinois.
- Chau, P.Y., and Y.K. Leung. 1978. Inhibitory action of various plating media on the growth of certain Salmonella serotypes. J. Appl. Bacteriol. 45:341-345.
- Chen, K.Y. and F.R. Bowerman. 1974. Mechanisms of leachate formation in sanitary landfills. In T. F. Yen (ed.) Recycling and Disposal of Solid Wastes, Industrial, Agricultural, Domestic. Ann Arbor Science, Ann Arbor, Michigan.
- Chian, E.S.K., and DeWalle, F.B. 1975. Compilation of methodology for measuring pollution parameters of sanitary landfill leachate, E.P.A. 600/3-75-011, October, 1975.
- Chian, E.S.K. and F.B. DeWalle. 1977. Evaluation of Leachate Treatment. Vol. 1, Characterization of Leachate. Municipal Environmental Research Laboratory, U. S. Environmental Protection Agency, Report No. EPA-600/2-77-186a. September, 1977.

- Cherry, W.B., J.B. Hanks, B.M. Thomason, A.M. Murlin, J.W. Biddle, and J.M. Croom. 1972. Salmonellae as an index of pollution of surface waters. Appl. Microbiol. 24:334-340.
- Coe, J.J. 1970. Effect of solid waste disposal on ground water quality. J. Amer. Water Works Assoc. 62:776-783.
- Communicable Disease Center. 1974. Etiologic agents classified on the basis of Hazard. U.S. Department of Health, Education, and Welfare. Atlanta, Georgia. p. 13.
- Conant, N.F., D.T. Smith, R.D. Baker, and J.L. Callaway. 1971. Manual of clinical mycology. W.B. Saunders Co., Philadelphia.
- Cook, H.A. 1966. Microbiological and chemical investigation of seepage from a sanitary landfill. Master's Thesis. West Virginia University.
- Cook, H.A., D.L. Cromwell, and H. A. Wilson. 1967. Microorganisms in household refuse and seepage water from sanitary landfills. Proc. W. Va. Acad. Sci. 39:107-114.
- Cooper, R.C., J.L. Potter, and C. Leong. 1974. Virus survival in solid waste treatment systems. pp. 218-232. In J.F. Milana, Jr. and B.P. Sagik (eds.) Virus Survival in Water and Wastewater Systems, Water Resources Symposium, Number 7. Center for Research in Water Resources, University of Texas, Austin.
- Cowan, S.T. 1977. Cowan and Steel's manual for the identification of medical bacteria. 2nd ed. Cambridge University Press, New York.
- Coyle, M.B. and L.S. Tompkins. 1980. Corynebacteria pp. 131-138. In E.H. Lennette, A. Balows, W.J. Hausler, Jr. and J.P. Truant (eds). Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
- Craun, G.F. 1979. Waterborn disease - a status report emphasizing outbreaks in ground water system. Ground Water 17:183-191.
- Craun, G.F. and L.J. McCabe. 1973. Review of the causes of waterborne disease outbreaks. J. Amer. Water Works Assoc. 65:74-84.

- Craun, G. F., L. J. McCabe, and J. M. Hughes. 1976. Water-borne disease outbreaks in the United States - 1971-1974. *J. Amer. Water Works Assoc.* 68:4106-4110.
- Cromwell, D.L. 1965. Identification of microflora present in sanitary landfills. Masters Thesis, West Virginia University.
- Cruickshank, R., J.P. Duguid, B.P. Marmion, and R.H.A. Swain. 1975. Medical microbiology, the practice of medical microbiology, 12th ed., Vol 2. Churchill Livingstone, Edinburgh.
- Davis, B.D., R. Dulbecco, H.N. Eisen, E.S. Ginsberg, W.B. Wood, Jr., and M. McCarty. 1973. Microbiology including immunology, and molecular genetics, 2nd ed. Harper and Row, New York.
- Davis, B.D., R. Dulbecco, H.N. Eisen, and E.S. Ginsberg. 1980. Microbiology including immunology, and molecular genetics, 3rd ed. Harper and Row, Hagerstown.
- Deibel, R.H. and C.F. Niven. 1958. Microbiology of meat curing. I. The occurrence and significance of a motile microorganism of the genus Lactobacillus in ham curing brines. *Appl. Microbiol.* 6:323-327.
- Deutsch, M. 1965. Natural controls involved in shallow aquifer contamination. *Ground Water* 3:37-40.
- Dobson, A.L. 1964. Microbial decomposition investigations in sanitary landfills. Ph.D. Dissertation. West Virginia University, Morgantown, West Virginia.
- Dowell, V.R., Jr. and T.M. Hawkins. 1976. Laboratory methods in anaerobic bacteriology. CDC Laboratory Manual. Public Health Service Center for Disease Control, Atlanta, Georgia.
- Dudley, D.J., M.N. Guentzel, M.J. Ibarra, B.E. Moore, and B.P. Sagik. 1980. Enumeration of potentially pathogenic bacteria from sewage sludges. *Appl. Environ. Microbiol.* 39:118-126.
- Duncan, C.L., R.G. Labbe, and R.R. Reich. 1972. Germination of heat and alkali altered spores of Clostridium perfringens type A by lysozyme and an initiation protein. *J. Bacteriol.* 109: 550-559.

- Duncan, C.L., and S.M. Harmon. 1974. Clostridium perfringens, pp. 437-447. In M. L. Speck (ed.) Compendium of methods for the microbiological examination of foods. American Public Health Association Intersociety/Agency Committee on Microbiological Methods for Foods.
- Edwards, P.R. and W.H. Ewing. 1962. Identification of Enterobacteriaceae. Burgess Publishing Co., Minneapolis, Minnesota.
- Eliassen, R. 1942. Decomposition of Landfills. Amer. J. Publ. Health. 32:1029-1037.
- Elliot, L.F., and J.R. Ellis. 1977. Bacterial and viral pathogens associated with land application of organic wastes. J. Environ. Qual. 6:245-251.
- Emmons, C.W., C.H. Binford, J.P. Utz, and K.J. Kwon-chung. 1977. Medical mycology, 3rd ed. Lea and Febiger, Philadelphia.
- Engelbrecht, R.S., M.J. Weber, P. Amirhor, D.H. Foster, and D. LaRossa. 1974. Biological properties of sanitary landfill leachate, p. 201-207. In J.F. Milana, Jr. and B.P. Sagik (eds.) Virus survival in water and wastewater systems, water resources symposium number seven. Center for Research in Water Resources, University of Texas, Austin.
- Esco/Greenleaf. 1972. Solid waste handling and disposal in multistory buildings and hospitals. Vols I and III from U.S. Government Printing Office, Washington. Vols II and IV from National Technical Information Service, Springfield.
- Ewing, W.H. and W.J. artin. 1974. Enterobacteriaceae. pp. 189-221. In E.H. Lennette, E.H. Spaulding, and J.P. Truant (eds.) Manual of clinical microbiology, 2nd ed. American Society for Micro biology, Washington, D.D.
- Facklam, R.R., J.F. Padula, L.G. Thacker, E.C. Wortham, and N.J. Sconyers. 1974. Presumptive identification of Group A, B, and D Streptococci. Appl. Microbiol. 27:107-113.
- Facklam, R.R., J.F. Padula, E.C. Wortham, R.D. Cooksey, and H.A. Roundtree. 1979. Presumptive identification of Group A, B, and D streptococci on agar plate media. J. Clin. Microbiol. 9:665-672.

- Fair, G.M., J.C. Geyer, and D.A. Okum. 1968. Water and wastewater engineering. Vol. 2. John Wiley and Sons, Inc., New York.
- Faulkner, G.L. and C.A. Pascale. 1975. Monitoring regional effects of high pressure injection of industrial waste water in a limestone aquifer. *Ground Water* 13:197-208
- Feachem, R. 1975. An improved role for faecal coliform to faecal streptococci ratios in the differentiation between human and non-human pollution sources. *Water Res.* 9:689-690.
- Fetter, Jr., C.W. 1980. Applied hydrology. Charles E. Merrill Publishing Co., Columbus. pp. 34-35, 165-166.
- Finegold, S.M., W.J. Martin, and E.G. Scott. 1978. Bailey and Scott's diagnostic microbiology, 5th ed. C.V. Mosby Co., St. Louis.
- Foster, D.H., and R.S. Engelbrecht. 1973. Microbial hazards in disposing of wastewater on soil, p. 247-270. In W.E. Sopper and L.T. Kardos (eds.) Recycling treated municipal wastewater and sludge through forest and cropland. The Pennsylvania State Univer. Press, University Park.
- Foster, J.W. and R.H. Davis. 1966. A methane-dependent coccus, with notes on classification and nomenclature or obligate, methane-utilizing bacteria. *J. Bacteriol.* 91:1924-1931.
- Fournelle, H.J., E.K. Day, and W.B. Page. 1957. Experimental ground water pollution at Anchorage, Alaska. *Public Health Reports.* 72:203-209.
- Fusillo, M.H. and D.L. Weiss. 1959. Quantitative estimation of streptococcal deoxyribonucleases. *J. Bact.* 78:520-522.
- Geldreich, E.E. 1972. Waterborne pathogens, pp. 207-241. In R. Mitchell (ed.) Water pollution microbiology, chapt. 9. John Wiley & Sons, Inc., New York.
- Geldreich, E.E. 1976. Fecal coliform and fecal streptococcus density relationships in waste discharges and receiving waters. *Crit. Rev. Environ. Control.* 6:349-369.

- Geldreich, E.E., H.F. Clark, and C.B. Huff. 1964. A study of pollution indicators in a waste stabilization pond. *Water Poll. Control Fed.* 36:1372-1379.
- Geldreich, E.E., B.A. Kenner, and P.W. Kabler. 1964. The occurrence of coliforms, fecal coliforms, and streptococci on vegetation and insects. *Appl. Microbiol.* 12:63-69.
- Geldreich, E.E., L.C. Best, B.A. Kenner, and D.J. Van Donsel. 1968. The bacteriological aspects of storm water pollution. *Jour. Water Poll. Control Fed.* 40:1861-1872.
- Geldreich, E.E., and B.A. Kenner. 1969. Concepts of fecal streptococci in stream pollution. *Jour. Water Poll. Control Fed.* 41:R336-R352.
- Glottzbecker, R.A. 1974. Presence and survival in landfill leachates and migration through soil columns of bacterial indicators of fecal pollution. Masters thesis, University of Cincinnati.
- Glottzbecker, R.A. and A.L. Novello. 1975. Poliovirus and bacterial indicators of fecal pollution in landfill leachates. *News of Environmental Research in Cincinnati*, Jan. 31, 1975. U.S.E.P.A. Cincinnati.
- Goff, J.H., T.J. Claydon, and J.J. Iandolo. 1972. Revival and subsequent isolation of heat-injured bacteria by a membrane filter technique. *Appl. Microbiol.* 23:857-862.
- Guentzel, M. N. 1978. Potential impact on water resources of bacteria pathogens in wastewater applied to land, pp.180-194. In B.P. Sagik and C.A. Sorber (eds.) Risk assessment and health effects of land application of municipal wastewater and sludges. Center for Applied Research and Technology, The Univ. of Texas at San Antonio, Texas, 78285. Dec. 12-14, 1977.
- Gross, K.C., M.P. Houghton, and L.B. Senterfit. 1979. Use of rapid latex kit for the identification of group F streptococci (*Streptococcus anginosus*). *Abstr. Annual Meeting of ASM*, 1979. p. 338.
- Hanks, T.G. 1967. Solid waste/disease relationships, a literature survey. U. S. Department of Health, Education, and Welfare. Public Health Service, Cincinnati.

- Harmon, S.M.D., D.A. Kautter, and J.T. Peeler. 1971. Improved medium for enumeration of Clostridium perfringens. Appl. Microbiol. 22: 688-692.
- Harmon, S.M., and D.A. Kautter. 1977. Recovery of clostridia and catalase-treated plating media. Appl. Environ. Microbiol. 33: -770.
- Harrigan, W.F. and M.E. McCance. 1976. Laboratory methods in food and dairy microbiology. Academic Press, London.
- Hartman, P.A., P.S. Hartman, and W.W. Lanz. 1975. Violet red bile agar for stressed coliforms. Appl. Microbiol. 29:537-539.
- Harvey, R.W.S., and T.H. Price. A review. Principles of Salmonella isolation. J. Appl. Bacteriol. 46:27-56.
- Heinmets, F. 1953. Reactivation of ultraviolet inactivated Escher-ichia coli by pyruvate J. Bacteriol. 66:455-457.
- Heinmets, F., W.W. Taylor, and J.J. Lehman. 1954a. The use of metabolites in the restoration of the viability of heat and chemically inactivated Escherichia coli. J. Bacteriol. 67: 5-12.
- Heinmets, F., J.J. Lehman, W.W. Taylor, and R.H. Kathan. 1954b. The study of factors which influence metabolic reactivation of the ultraviolet inactivated Escher-ichia coli. J. Bacteriol. 67:511-522.
- Hendricks, C.W. 1974. Sorption of heterotrophic and enteric bacteria to glass surfaces in the continuous culture of river water. Applied Microbiology. 28:572-578.
- Heukelkian, H., and M. Albanese. 1956. Enumeration and survival of human tubercle bacilli in polluted waters. II. Effect of sewage treatment and natural purification. Sewage Ind. Wastes. 28:1094-1102.
- Hoadley, A.W. and S.M. Goyal. 1976. Public health implications of the application of wastewaters to land. pp.101-132. In R.L. Sanks and T. Asano (eds.) Land treatment and disposal of municipal and industrial wastewater.
- Hughes, D.E. 1980. What is anaerobic digestion? An overview. In D.A. Stafford, B.I. Wheatly, and D.E. Hughes (eds.) Anaerobic Digestion, Proceedings of the First International Symposium on Anaerobic Digestion at the University College, Cardiff, Wales in 1979. Applied Science Publishers, LTD, London.

- Hutchinson, M. 1974. Microbiological aspects of groundwater pollution. pp. 167-202. In J.A. Cole (ed.) Ground water pollution in Europe. Water Information Center, Inc., Fort Washington, New York.
- Iandolo, J.J., C.W. Clark, L. Bluhm, and Z.J. Ordal. 1965. Repression of Staphylococcus aureus in associative culture. Appl. Microbiol. 13:646-649.
- Jackson, A.G., and D.R. Streng. 1976. Gas and leachate generation in various solid waste environments. pp. 83-91. In E. J. Gentelli and J. Cirello (eds.) Gas and leachate from landfills, formation, collection, and treatment. Proceedings of the Hazardous Waste Research Symposium EPA-600/9-76-004, Environmental Protection Agency, Municipal Environmental Agency, Cincinnati, Ohio.
- Jensen, F.E. 1954. Presence and destruction of tubercle bacilli in sewage. Bull. WHO 10:171-179.
- Johnson, M. and D.N. Urie. 1977. Ground-water pollution aspects of land disposal of sewage from remote recreation areas. Ground Water. 14:403-410.
- Kabler, P. 1959. Removal of pathogenic microorganisms by sewage treatment processes. Sewage Ind. Wastes. 31:1373-1382.
- Kafel, S. and F.L. Bryan. 1977. Effects of enrichment media and incubation conditions on isolating Salmonellae from ground-meat filtrate. Appl. Environ. Microbiol. 24:285-291.
- Karung, J.R. and Yegin. 1954. Medium for maintenance and conversion of Histoplasma capsulatum to yeast-like phase. Amer. J. Clin. Path. 24:505-508.
- Kanner, B.A., and R.F. Clark. 1974. Detection and enumeration of Salmonella and Pseudomonas aeruginosa. J. Water Poll. Contr. Fed. 46:2163-2171.
- Kawick, D.E. and C.F. Corbo. 1980. Viruses in Groundwater. Environ. Sci. and Tech. 14:1290-1297.
- Kinnel, G.E. and G.C. Braide. 1974. Leachate plumes in a highly permeable aquifer. Ground Water. 12:388-393.

- Klein, D.A., and S. Wu. 1973. Stress: A factor to be considered in heterotrophic microorganism enumeration from aquatic environments. *Appl. Microbiol.* 27:429-431.
- Koch, A.L. 1981. Growth measurement. pp. 179-207. In P. Gebhardt, R.E. Murray, R.N. Costilow, G.W. Nester, W.A. Wood, N.R. Krieg, and G.B. Phillips (eds.) *Manual of methods for general microbiology*. American Society for Microbiology, Washington, D.C.
- Koneman, E.W., G.D. Roberts, and S.E. Wright. 1978. *Practical Laboratory Mycology*, 2nd Ed. Williams and Wilkins Co., Baltimore.
- Koneman, E.W., S.D. Allen, V.R. Dowell Jr., and H.M. Sommers. 1979. Color atlas and textbook of diagnostic microbiology. J.B. Lippincott Co., Philadelphia.
- Kugelman, I.J. and J.S. Jeris. 1981. Anaerobic Digestion, Chapter 7 In W. Eckenfelder and C. Santhanam (eds.) *Sludge Treatment*. Marcel Dekker, New York.
- Larone, D.H. 1976. *Medically important fungi, a guide to identification*. Harper and Row Publishers, Hagerstown, Maryland.
- Leadbetter, E.R. and J.W. Foster. 1958. Studies on some methane-utilizing bacteria. *Arch. Mikrobiol.* 30:91-118. In S. Aaronson (ed.) *Experiental microbiology ecology*. Academic Press, New York. 1970.
- Lehninger, A.L. 1975. *Biochemistry* 2nd Ed. Worth Publishers, Inc., New York, New York. pp.417-441.
- Lennette, E.H., E.H. Spaulding, and J.P. Truant. 1974. *Manual of clinical microbiology*. 2nd ed. American Society for Microbiology, Washington, D.C.
- Lennette, E.H., A. Balows, W.H. Hausler, Jr., and J.P. Truant. 1980. *Manual of clinical microbiology*. 3rd ed. American Society for Microbiology, Washington, D.C.
- Lin, T.H. 1966. Acid and gas production from sanitary landfills. Ph.D. Dissertation Department of Civil Engineering, University of West Virginia, Morgantown, West Virginia.

- Love, G.J., E. Tompkins, and W.A. Galke. 1975. Potential health impacts of sludge disposal on the land. pp. 204-213. In Proceedings of the 1975 National Conference of Municipal Sludge Management and Disposal. U. S. Environmental Protection Agency, Anaheim, California, August 18-20.
- Mann, J.F. 1976. Wastewaters in the vadose zone of arid regions: Hydrologic interactions. Ground Water. 14:367-373.
- Marshall, K.C. 1979. Growth at interfaces. pp. 281-290. In M. Shile (ed.) Strategies of microbial life in extreme environments. Berlin, Dahlem Konferenzen.
- Marshall, K.C. 1980. Microorganisms and interfaces. Bio-science, 30:246-249.
- McCabe, L.J. and G.F. Craun. 1975. Status of waterborne disease in the United States and Canada. J. Amer. Water Works Assoc. 67:95-98.
- McCarty, P.L. 1964. The methane fermentation. In Principles and applications in aquatic microbiology. H. Heukelekian and N.C. Dondero (eds.). John Wiley, New York.
- MacFaddin, J.F. 1976. Biochemical tests for identification of medical bacteria. The Williams and Wilkins Co., Baltimore.
- McKinley, G.A., D.J. Fagerberg, C.L. Quarles, B.A. George, D.E. Wagner, and L.D. Rollins. 1980. Incidence of Salmonellae in fecal samples of production swine and swine at slaughter plants in the United States in 1978. Appl. Environ. Microbiol. 40: 562-566.
- McKinney, R.E., H.E. Langley and H.D. Tomlinson. 1958. Survival of Salmonella typhosa during anaerobic digestion. Sewage and Industrial Wastes. 30:1469-1473.
- Methods for chemical analysis of water and wastes. 1974. EPA-625/6-74-002a. Environmental Monitoring and Support Laboratory. Environmental Research Center, Cincinnati, Ohio, 45208.
- Malina, J.E., O.C. Braide, and T.D. Hinesly. 1972. Observation on bactericidal properties of digested sewage sludge. Environ. Sci. Technol. 6:446-450.

- Moss, E.S. and A.L. McQuown. 1969. Atlas of medical mycology. The Williams and Wilkins Co., Baltimore.
- Mossel, D.A.A., and M. A. Ratto. 1970. Rapid detection of sublethally impaired cells of Enterobacteriaceae in dried foods. Appl. Microbiol. 20:273-275.
- Niesson, W.R. and S.H. Chansky. 1970. The nature of refuse, pp. 1-24. In Proceedings of 1970 National Incineration Conference.
- Norris, F.S., and B.G. Young. 1978. Guidelines for defining and disposing of medical waste. Aviat. Spac Environ. Med. 49: 81-85.
- Novello, A.L. 1974. Poliovirus Survival in Landfill Leachate and Migration through Soil Columns. Master's Thesis. University of Cincinnati.
- Padhye, A.A. and L. Ajello. 1980. Fungi causing Eumycotic mycetomas. pp. 595-601. In E.H. Lennette, A. Balows, W.J. Hausler, Jr., and J.P. Truant (eds.) Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
- Pavlova, M.T., F.T. Brezenski, and W. Litsky. 1972. Evaluation of various media for isolation, enumeration and identification of fecal streptococci from natural sources. Health Lab. Sci. 9:289-298.
- Pavoni, J.L., J.E. Heer Jr., and D.J. Hagerty. 1975. Handbook of Solid Waste Disposal, Materials and Energy Recovery. Van Nostrand Reinhold Company, New York.
- Paynter, M.J.B. and R.E. Hungate. 1968. Characterization of Methanobacterium mobilis, sp. n., isolated from the bovine rumen. J. Bact., 95:1943-1951.
- Peterson, M.L., and F.J. Stutzenberger. 1969. Microbiological evaluation of incinerator operations. Appl. Microbiol. 18: 8-13.
- Peterson, M.L., and A.J. Klee. 1971. Studies on the detection of Salmonellas in municipal solid waste and incinerator residue. Int. J. Environ. Studies. 2:123-132.
- Peterson, M.L. 1971a. Pathogens associated with solid waste processing. Open-file Report SW-49r, U. S. Environmental Protection Agency.

- Peterson, M.L. 1971b. The occurrence and survival of viruses in municipal solid waste. Doctoral Dissertation, University of Michigan.
- Peterson, M.L. 1973. Methods for bacteriological examination of solid waste and waste effluents. Part III. Microbiological Methods, pp.2-19. In D.F. Bender, M.L. Peterson, and H. Sterli (eds.) Physical, chemical and microbiological methods of solid waste testing. National Environmental Research Center, USEPA, Cincinnati, Ohio, EPA-6700-73-01.
- Peterson, M.L. 1974. Soiled disposable diapers: a potential source of viruses. Amer. J. Pub. Health. 64:912-914,
- Pfeffer, J.J. 1974. Reclamation of energy from organic waste. EPA-1-670/ 2-74-016. Solid and Hazardous Waste Research Laboratory, National Environmental Research Center, Cincinnati, Ohio.
- Pohland, F.G. 1972. Landfill stabilization with leachate recycle, Annual Progress Report, EPA No. EP-00658. Cited and described by J.L. Pavoni, J.E. Heer, Jr., and D.J. Hagerty, Handbook of Solid Waste Disposal, Materials and Energy Recovery, Van Nostrand Co., Cincinnati, Ohio.
- Pohland, F.G. 1972. Landfill stabilization with leachate recycle, interim progress report, Grant GR 00658-01, Solid Waste Research Division, USEPA.
- Pohland, F.G. 1975. Sanitary landfill stabilization with leachate recycle and residual treatment. Environmental Protection Agency Report 600/2-75-043, Cincinnati, Ohio.
- Postgate, J.R. 1967. Viability measurements and the survival of microbes under minimum stress. Adv. in Microbial Physiol. 1:1-23
- Qasim, S.R. 1965. Chemical Characteristics of Seepage Water from Simulated Landfills, Doctorial Dissertation, Department Civil Engineering, University of West Virginia, Morgantown.
- Ray, S., J.J. Jasecki, and F.F. Rusto. 1971. Effect of rehydration on recovery, repair, and growth of injured freeze-dried Salmonella anatum. Appl. Microbiol. 22:184-189.

- Ray, B., and M.L. Speck. 1972a. Repair of injury induced by freezing Escherichia coli as influenced by recovery medium. Appl. Microbiol. 24:258-263.
- Ray, B., and M.L. Speck. 1972b. Metabolic process during the repair of freeze injury in Escherichia coli. Appl. Microbiol. 24:585-590.
- Ray, B., and M.L. Speck. 1973. Discrepancies in the enumeration of Escherichia coli. Appl. Microbiol. 25:494-498.
- Read, R.B. Jr., and A.L. Reyes. 1968. Variation in plating efficiency of Salmonellae on eight lots of brilliant green agar. Appl. Microbiol. 16:746-748.
- Riley, R.D., R.G. Benedict, R.W. Seabloom, and D.A. Carlson. 1977. Chemical and microbiological studies on leachates from a waste tip. J. Appl. Bacteriol. 42:285-288.
- Rippon, J.W. 1974. Medical mycology, the pathogenic fungi and the pathogenic actinomycetes. W.B. Saunders Company, Philadelphia.
- Roebeck, G.G. 1969. Microbial problems in Ground Water. Ground Water 7:33-35.
- Rose, R.E., and W. Litskey. 1965. Enrichment procedure for use with the membrane filter for the isolation and enumeration of fecal streptococci in water. Appl. Microbiol. 13:106-108.
- Rose, R.E., E.E. Geldreich, and W. Litskey. 1975. Improved membrane filter method for fecal coliform analysis. Appl. Microbiol. 29:932-936.
- Rudolf, W., L.L. Falk, and R.A. Ragotskie. 1950. Literature review on the occurrence and survival of enteric, pathogenic and relative organisms in soil, water, sewage, and sludges and on vegetation. I. Bacterial and virus diseases. Sewage Ind. Wastes. 22:1261-1281.
- Rudolfs, W., L.L. Falk, and R.A. Ragotskie. 1951a. Contamination of vegetables grown in polluted soil. Sewage Ind. Wastes. 23:253-268.
- Rudolfs, W., L.L. Falk, and R.A. Ragotskie. 1951b. Contamination of vegetables grown in polluted soil. IV. Bacterial contamination. Sewage Ind. Wastes 23:739-751.

- Rule, J.H. 1979. Municipal landfill leachate in the ground and surface water, Chesapeake, Virginia: Heavy Metals. *J. Environ. Health.* 42:60-63.
- Runyon, E.H., A.G. Karlson, G.P. Kubica, and L.G. Wayne. 1974. *Mycobacterium*. pp. 148-174. In E.H. Lemette, E.H. Spaulding, and J.P. Truant (eds.) *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
- Runyon, E.H., A.G. Karlson, G.P. Kubica, and L.G. Wayne. 1980. *Mycobacterium*. In E.H. Lennette, A. Balows W.J. Hausler, Jr., and J.P. Truant (eds.) *Manual of clinical microbiology*, 3rd ed. American Society for Microbiology, Washington, D.C.
- Salvato, J.A., W.G. Wilkie, and B.E. Mead. 1971. Sanitary landfill - leachate prevention and control. *J. Water Poll. Control Fed.* 43:2084-2100.
- Sagik, B.P., S.M. Duboise, and C.A. Sorber. 1980. health risks associated with microbial agents in municipal sludge. pp. 15-46. In G. Bitton, B.L. Damron, G.T. Edds, and J.M. Davidson. *Sludge -- health risks of land application*. Ann Arbor Science, Ann Arbor, Michigan.
- Scarpino, P.V. 1971. Bacterial and viral analysis of water and waste water. In L.L. Ciaccio (ed.) *Water and water pollution handbook*. Vol II, Marcel Dekker New York. Chap. 13, pp. 639-761.
- Scarpino, P.V. 1975. Human enteric viruses and bacteriophages as indicators of sewage pollution. pp. 49-6. In A.L.H. Gameson (ed.) *Discharge of sewage from sea outfalls*. Pergamon Press, Oxford, England.
- Scherago, M. 1972. Literature Search: Pathogens in and-fill leachate and possible contribution of disposable diapers. National Technical Information Service, . S. Department of Commerce, Springfield, Virginia.
- Schwenner, D.L., F.F. Busta, and M.L. Speck. 1971. Injury of bacteria by sanitizers. *Appl. Microbiol.* 21:4-45.
- Schwartzbaugh, J.T., R.L. Santrich, Jr., and G. Sahel. 1978. Evaluation of landfill municipal and industrial solid wastes. Research #68-03-2128. Municipal Environmental Research Laboratory, U.S. Environmental Protection Agency.

- Shaub, S.A. and C.A. Sorber. 1977. Virus and bacteria removal from wastewater by rapid infiltration through soil. *Appl. Environ. Microbiol.* 33:609-619.
- Silvia-Hunter, M. and B.H. Cooper. 1974. Medically important yeasts. pp. 491-507. In E.H. Lennette, E.H. Spaulding, and J.P. Truant (eds.) *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
- Silvia-Hunter, M. and B.H. Cooper. 1980. Yeasts of medical importance. pp. 562-576. In E.H. Lennette, W.J. Hausler, Jr., and J.P. Truant (eds.) *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
- Sinskey, T.J. and G.J. Silverman. 1970. Characterization of injury incurred by *Escherichia coli* upon freeze-drying. *J. Bacteriol.* 101:429-437.
- Smith, L.D.S., and V.R. Dowell, Jr. 1974. *Clostridium*, pp. 376-387. In E.H. Lennette, E.H. Spaulding, and J.P. Truant (eds.) *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D. C.
- Smolka, L.R., F.E. Nelson, and L.M. Kelley. 1974. Interaction of pH and NaCl on enumeration of heat-stressed *Staphylococcus aureus*. *Appl. Microbiol.* 27:443-447.
- Sobsey, M.D., C. Wallis, and J.L. Melnick. 1974. Development of methods for detecting viruses in solid waste landfill leachates. *Appl. Microbiol.* 28:232-238.
- Sobsey, M.D., C. Wallis, and J.L. Melnick. 1975. Studies on the survival and rate of enteroviruses in an experimental model of a municipal solid waste landfill and leachate. *Appl. Microbiol.* 30:965-974.
- Sobsey, M.D. 1978. Field survey of enteric viruses in solid waste landfill leachates. *Amer. J. Pub. Health.* 68:858-864.
- Society of American Bacteriologists, Committee on Bacteriological Testing. 1957. *Manual of microbiological methods*. McGraw-Hill Book Co., New York.
- Solid Waste Disposal Act. Title II of Public Law 89-272, 89th Congress, S.306, October 20, 1965. Washington, D.C. Public Law 91-613, S.R. 11833, Oct. 24, 1970.

- Sonoma County Department of Public Works and EMCOM Associates. 1972. First Interim Annual Report, Vol. I, Solid Waste Disposal Demonstration Grant Project G-06-EC-00351. Cited and described by J.L. Pavoni, J.E. Heer, Jr., and D.J. Hagerty, Handbook of Solid Waste Disposal, Materials, and Energy Recovery. Van Nostrand Co., Cincinnati, Ohio.
- Sonoma County Department of Public Works and EMCOM Associates. 1973. Second Interim Annual Report, Vol. II, Solid Waste Disposal Demonstration Grant Project G-06-EC-00351. Cited and described by J.L. Pavoni, J.E. Heer, Jr., and D.J. Hagerty, Handbook of Solid Waste Disposal, Materials, and Energy Recovery. Van Nostrand Co., Cincinnati, Ohio.
- Sparks, S. 1981. Heat recovery from incineration of solid waste from hospitals. Publ. Health Reports 96:105-110.
- Speck, M.L., B. Ray, and R.B. Read, Jr. 1975. Repair and enumeration of injured coliforms by a plating procedure. Appl. Microbiol. 28:549-550.
- Stanforth, R.R. Ham, M. Anderson, and R. Stegmann. 1979. Development of a synthetic municipal landfill leachate. J. Water Poll. Control Fed. 51:1965-1975.
- Stanier, R.Y., E.A. Adelberg, and J.L. Ingraham. 1976. The microbial world. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.
- Stone, R. 1975. Landfill disposal of liquid sewage sludge. Amer. Soc. Civil Eng. (HE1). 101:91-105.
- Strayer, R.F. and J.M. Tiedje. 1978. Application of the fluorescent-antibody technique to the study of a methanogenic bacterium in lake sediments. Appl. Environ. Microbiol., 35:192-198.
- Strong, D.R. 1976. The effects of the disposal of industrial waste within a sanitary landfill environment. pp.51-70. In W.H. Fuller (ed.) Accident management by land disposal. Proceedings of the Hazardous Waste Research Symposium EPA-600/9-76-015. Environmental Protection Agency, Municipal Environmental Research Laboratory, Cincinnati, Ohio.

- Techobanoglous, G., H. Thiese, and R. Eliassen. 1977. Solid wastes, engineering principles and management issues. McGraw-Hill Book Company, New York.
- Temple, K.L., A.K. Camper, and G.A. McFeters. 1980. Survival of two enterobacteria in feces Buried in soil under field conditions. 40:794-797.
- Thomason, B.M., J.W. Biddle, and W.B. Cherry. 1975. Detection of Salmonella in the Environment. Appl. Microbiol. 30:764-767.
- Thomason, B. M., D. J. Dodd, and W. B. Cherry. 1977. Increased recovery of Salmonella from environmental samples enriched with buffered peptone water. Appl. Environ. Microbiol. 34:270-273.
- Tierney, J.T., R. Sullivan, E.P. Larkin. 1977. Persistence of poliovirus 1 in soil and on vegetables grown in soil previously flooded with inoculated sewage sludge or effluent. Appl. Microbiol. 33:109-113.
- Treagan, L. and L. Pulliam. 1981. Medical microbiology laboratory procedures. W.B. Saunders Co., Philadelphia.
- U.S. Environmental Protection Agency. 1979. Methods for chemical analysis of water and wastes. National Environmental Research Center, USEPA, Cincinnati, Ohio, EPA600/4-79-020.
- Van Donsel, D.J., and E.P. Larkin. 1977. Persistence of Mycobacterium bovis BCG in soil and on vegetables spray-irrigated with sewage effluent and sludge. J. Food Prot. 40:160-163.
- Vaughn, J.W., E.F. Landry, L.J. Baranovsky, L.A. Beckwith, C.M. Dahl, and W.C. Delibes. 1978. Survey of human virus occurrence in wastewater-recharged ground water on Long Island. Appl. Environ. Microbiol. 33:109-113.
- Veetel, G.C. 1976. Procedures for the isolation and identification of Mycobacteria. Center for Disease Control. U. S. Health, Education, and Welfare. Atlanta, Ga.
- Walker, W.B. 1973. Where have all the toxic chemical gone? Ground Water, 11:11-28.
- Walker, W.B., G. Veetel, and J.C. Baranovsky. 1977. Where will the toxic waste come from? where it should go. Sci. Prog. 118:33-42.

- Walsh, J.J., and R.N. Kinman. 1979. Leachate and gas production under controlled moisture conditions. pp. 41-57. In M.P. Wanielista and J.S. Taylor (eds.) Proceedings of the fifth annual research symposium municipal solid waste: land disposal. Solid and Hazardous Waste Research Division. USEPA, Cincinnati, Ohio.
- Walsh, J.J., R.N. Kinman, and J.I. Rickabaugh. 1981. Leachate and gas from municipal solid waste landfill simulators. pp. 67-93. In D.W. Shultz (ed.) Land disposal: municipal solid waste. Proceedings of the seventh annual research symposium at Philadelphia, Pennsylvania, March 16-18, 1981. Municipal Environmental Research Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio.
- Wang, W.L.L., S.G. Dunlap, and R.G. DeBoer. 1956. The survival of Shigella in sewage. I. An effect of sewage and fecal suspensions on Shigella flexneri. Appl. Microbiol. 4:34-38.
- Ware, S.A. 1980. A survey of pathogen survival during municipal solid waste and manure treatment processes. EPA-600/8-80-034. Municipal Environmental Research Laboratory, Office of Research and Development, U. S. Environmental Protection Agency, Cincinnati, Ohio.
- Washington J.A. (ed.) 1981. Laboratory procedures in clinical microbiology. Springer-Verlag, New York.
- Watson, D.C., and A.P. Walker. 1978. A modification of brilliant green agar for improved isolation of Salmonella. J. Appl. Bacteriol. 45:195-204.
- Weaver, L. 1964. Refuse disposal, its significance. Ground Water 2:26-30.
- Weaver, S.J., H.W. Tate, and D.G. Hollie. 1972. The identification of unusual pathogenic gram-negative bacteria (Elizabeth O. King). Public Health Service, Center for Disease Control, Atlanta, Georgia.
- Wentz, L.G. and B.W. Catlin. 1957. Deoxyribonuclease activity of micrococci from clinical sources. J. Bact. 73:74-753.
- Wise, J. and E.F.G. Seeliger. 1979. Incidence of Listeria monocytogenes in nature. Appl. Microbiol. 28:24-27.

- Wellings, F.M., A.L. Lewis, C.W. Mountain, and L.V. Pierce. 1975. Demonstration of virus in groundwater after effluent discharge onto soil. *Appl. Microbiol.* 29: 751-757.
- Whittenbury, R., K.C. Philips, and J.F. Wilkinson. 1970. Enrichment, isolation and some properties of methane-utilizing bacteria. *J. Gen. Microbiol.* 61:205-218.
- Wilson, G.S., and A. Miles. 1975. Topley and Wilson's principles of bacteriology, virology, and immunity, 6th ed., Vol. 1. The Williams and Wilkins Co., Baltimore.
- Wigh, R.J. Interim summary report: Boone County field site - test cell 1. Municipal Environmental Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.
- Wigh, R.J. 1979. Boone County field site interim report, test cells 2A, 2B, 2C, and 2D. EPA-600/2-79-059 Municipal Environmental Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.
- Wolfe, R.S. 1970. Microbial formation of methane. *Adv. in microbial physiol.* 10:107-146.
- Wolf, P.L., B. Russell, and A. Shimoda. 1975. Practical clinical microbiology and mycology: techniques and interpretations. John Wiley and Sons, New York.
- Wu, S., and D.A. Klein. 1976. Starvation effects on *Escherichia coli* and aquatic bacterial responses to nutrient addition and secondary warning stresses. *Appl. Microbiol.* 31:216-220.
- Yeager, J.G., and R.T. O'Brien. 1979a. Enterovirus inactivation in soil. *Appl. Environ. Microbiol.* 29:694-701.
- Yeager, J.G., and R.T. O'Brien. 1979b. Structural Changes Associated with poliovirus inactivation in soil. *Appl. Environ. Microbiol.* 29:702-709.
- Zarogian, A.E. 1972. Ground-water pollution and sanitation landfills - a critical review. *Ground Water.* 10:3-13.
- Zarogian, A.E. and R.W. Nevers. 1978. Effect of environmental factors on survival of *Salmonella typhimurium* in soil. *J. Environ.* 9:333-337.

XI. APPENDICES

- A. HYDROLOGICAL DATA FROM THE SIX UNIVERSITY OF CINCINNATI LYSIMETERS
- B. MEDIA AND REAGENTS
- C. TEST PROCEDURES
- D. THE MEASUREMENT OF METHANE-UTILIZING AND METHANE-PRODUCING BACTERIA IN SOLID WASTE FROM THE BOONE COUNTY LANDFILL
- E. MICROBIAL PARAMETERS OF LEACHATE FROM LANDFILLS AND LYSIMETERS
- F. CHEMICAL PARAMETERS OF LEACHATE FROM LANDFILLS AND LYSIMETERS

A. HYDROLOGICAL DATA FROM THE SIX UNIVERSITY OF
CINCINNATI LYSIMETERS

TABLE A-1. HYDROLOGIC RECORD OF LYSIMETER UC-A (PART I)

Date	Monthly Liquid Exchange in Lysimeter			Moisture Retained in Cell	
	Water ^b Additions (month year)	Leachate Removed (liters)	Net Added +, Removed - (liters)	Kg ^c (liters)	Kg H ₂ O/ Kg Dry Refuse (35.1 kg)
Initial	— ^d	—	—	81.9	2.33
8/1978	—	2.9	- 2.9	79.0	2.25
9/1978	5.52	12.37	- 6.85	72.15	2.06
10/1978	11.2	10.81	+ .39	72.54	2.07
11/1978	11.2	10.97	+ .43	72.97	2.08
12/1978	11.2	13.51	- 2.31	70.66	2.01
1/1979	5.6	6.07	- .47	70.19	2.00
2/1979	11.2	9.67	+ 1.53	71.72	2.04
2 1/3/1979	16.8	15.0	+ 1.80	73.52	2.09
4/1979	16.8	17.2	- .4	73.12	2.08
5/1979	16.8	17.28	- .48	72.64	2.07
6/1979	11.2	11.78	- .58	72.06	2.05
7/1979	5.6	7.17	- 1.57	70.49	2.01
8/1979	5.6	6.0	- .4	70.09	2.00
9/1979	5.6	5.24	+ .36	70.45	2.01
10/1979	11.2	10.12	1.08	71.53	2.04
11/1979	11.2	10.05	+ 1.15	72.68	2.07
12/1979	11.2	4.06	+ 7.14	79.82	2.27

^aLysimeter UC-A contains Sewage Sludge.^bBoiled and cooled tap water was added weekly, and leachate was removed weekly.^cThe initial volume was obtained by subtracting the total dry weight from the total wet weight.^dNot determined.

TABLE A-1. HYDROLOGIC RECORD OF LYSIMETER UC-A^a (PART II)

Date	Monthly Liquid Exchange in Lysimeter ^b			Moisture Retained in Cell	
	Water ^b Additions	Leachate Removed	Net Added +, Removed -	Kg ^c	Kg H ₂ O/ Kg Dry Refuse (35.1 kg)
(Month/ year)	(liters)	(liters)	(liters)	(liters)	
1/1980	5.6	8.99	- 3.39	76.43	2.18
2/1980	11.2	9.23	+ 1.97	78.40	2.23
3/1980	16.8	15.34	+ 1.46	79.86	2.28
4/1980	16.8	17.31	- 0.51	79.35	2.26
5/1980	16.8	15.7	+ 1.1	80.45	2.29
6/1980	11.2	12.65	- 1.45	79.00	2.25
7/1980	5.6	7.25	- 1.65	77.35	2.20
8/1980	5.6	5.19	+ 0.41	77.76	2.22
9/1980	5.6	5.3	+ 0.3	78.06	2.22
10/1980	11.2	9.39	+ 1.81	79.87	2.28
11/1980	11.2	7.05	+ 4.15	84.02	2.39
12/1980	11.2	12.02	- 0.82	83.20	2.37
1/1981	5.6	8.09	- 2.49	80.71	2.30
2/1981	11.2	8.79	+ 2.41	83.12	2.37
3/1981	16.8	15.44	+ 1.38	84.50	2.41
4/1981	16.8	19.48	- 2.68	81.82	2.33
5/1981	16.8	16.07	+ 0.73	82.55	2.35
6/1981	11.2	12.13	- 0.93	81.62	2.33
7/1981	11.2	10.83	+ 0.37	81.99	2.34
8/1981	11.2	10.59	+ 0.61	82.60	2.35

^aLysimeter UC-A contains Sewage Sludge.

^bBoiled and cooled tap water was added weekly, and leachate was removed weekly.

^cThe initial volume was obtained by subtracting the total dry weight from the total wet weight. 378

• TABLE A-2. HYDROLOGIC RECORD OF LYSIMETER UC-B^a (PART I)

Date (Month/ year)	Monthly Liquid Exchange in Lysimeter			Moisture Retained in Cell	
	Water ^b Additions (liters)	Leachate Removed (liters)	Net Added +, Removed - (liters)	Kg ^c (37.2) (liters)	Kg H ₂ O/ Kg Dry Refuse (48.2 kg)
Initial	— ^d	—	—	37.20	0.77
8/1978	10.0	—	+ 10.0	47.20	0.98
9/1978	20.52	6.55	+ 13.97	61.17	1.27
10/1978	11.2	8.98	+ 2.22	63.39	1.32
11/1978	11.2	10.00	+ 1.2	64.59	1.34
12/1978	11.2	12.61	- 1.41	63.18	1.31
1/1979	5.6	5.23	+ 0.37	63.55	1.32
2/1979	11.2	9.02	+ 2.18	65.73	1.36
3/1979	16.8	14.73	+ 2.07	67.80	1.41
4/1979	16.8	17.53	- 0.73	67.07	1.39
5/1979	16.8	17.14	- 0.34	66.73	1.38
6/1979	11.2	11.29	- 0.09	66.64	1.38
7/1979	5.6	7.23	- 1.63	65.01	1.35
8/1979	5.6	5.89	- .29	64.72	1.34
9/1979	5.6	5.38	+ 0.22	64.94	1.35
10/1979	11.2	10.05	+ 1.15	66.09	1.37
11/1979	11.2	11.45	- .25	65.84	1.37
12/1979	11.2	11.51	- .31	65.53	1.36

^aLysimeter UC-B contains Sewage Sludge + Municipal Solid Waste.

^bBoiled and cooled tap water was added weekly, and leachate was removed weekly.

^cThe initial volume was obtained by subtracting the total dry weight from the total wet weight.

^dNot determined.

TABLE A-2. HYDROLOGIC RECORD OF LYSIMETER UC-B^a (PART II)

Date (Month/ year)	Monthly Liquid Exchange in Lysimeter			Moisture Retained in Cell	
	Water ^b	Leachate	Net	Kg ^c	Kg H ₂ O/
	Additions	Removed	Added +, Removed -		Kg Dry Refuse
	(Liters)	(liters)	(liters)	(liters)	(48.2 kg)
1/1980	5.6	8.1	- 2.5	63.03	1.31
2/1980	11.2	10.44	+ .76	63.79	1.32
3/1980	16.8	15.43	+ 1.37	65.16	1.35
4/1980	16.8	17.63	- 0.83	64.33	1.33
5/1980	16.8	15.96	+ 0.84	65.17	1.35
6/1980	1.2	12.73	- 1.53	63.64	1.32
7/1980	5.6	7.46	- 1.86	61.78	1.28
8/1980	5.6	5.71	- 0.11	61.67	1.28
9/1980	5.6	5.64	- 0.04	61.63	1.28
10/1980	11.2	11.79	- 0.59	61.04	1.27
11/1980	11.2	10.49	+ 0.71	61.75	1.28
12/1980	11.2	12.79	- 1.59	60.16	1.25
1/1981	5.6	7.23	- 1.63	58.53	1.21
2/1981	11.2	9.48	+ 1.72	60.25	1.25
3/1981	16.8	15.13	+ 1.67	61.92	1.28
4/1981	16.8	17.35	- 0.55	61.37	1.27
5/1981	16.8	15.84	+ 0.96	62.33	1.29
6/1981	11.2	15.21	- 4.01	58.32	1.21
7/1981	5.6	5.53	+ 0.07	58.39	1.21

^a Lysimeter UC-B contains Sewage Sludge + Municipal Solid Waste.

^b Boiled and cooled tap water was added weekly, and leachate was removed weekly.

^c The initial volume was obtained by subtracting the total dry weight from the total wet weight.

TABLE A-3. HYDROLOGIC RECORD OF LYSIMETER UC-C^a (PART I)

Date	Monthly Liquid Exchange in Lysimeter			Moisture Retained in Cell	
	Water ^b Additions	Leachate Removed	Net Added +, Removed -	Kg ^c	Kg H ₂ O/ Kg Dry Refuls!!Refuse
(Month/ year)	(liters)	(liters)	(liters)	(liters)	(liters)
Initial	-- ^d	--	--	24.3	0.51
8/1978	14.2	--	+ 14.2	38.50	0.80
9/1978	26.82	9.08	+ 17.74	56.24	1.17
10/1978	11.2	9.61	+ 1.59	57.83	1.20
11/1978	11.2	9.89	+ 1.31	59.14	1.23
12/1978	11.2	12.51	- 1.31	57.83	1.20
1/1979	5.6	4.86	+ .74	58.57	1.22
2/1979	11.2	9.11	+ 2.09	60.66	1.26
3/1979	16.8	14.64	+ 2.16	62.82	1.31
4/1979	16.8	15.25	+ 1.55	64.37	1.34
5/1979	16.8	17.99	- 1.19	63.18	1.32
6/1979	11.2	11.80	- .6	62.58	1.30
7/1979	5.6	7.29	- 1.69	60.89	1.27
8/1979	5.6	6.0	- .4	60.49	1.26
9/1979	5.6	5.33	+ .27	60.76	1.27
10/1979	11.2	10.07	+ 1.13	61.89	1.29
11/1979	11.2	10.58	+ 0.62	62.51	1.30
12/1979	11.2	11.34	- 0.14	62.37	1.30

^aLysimeter UC-C contains Municipal Solid Waste.

^bBoiled and cooled tap water was added weekly, and leachate was removed weekly.

^cThe initial volume was obtained by subtracting the total dry weight from the total wet weight.

^dNot determined.

TABLE A-3. HYDROLOGIC RECORD OF LYSIMETER UC-C^a (PART II)

Date (Month/ year)	Monthly Liquid Exchange in Lysimeter			Moisture Retained in Cell	
	Water ^b	Leachate	Net	Kg ^c	Kg H ₂ O/ Kg Dry Refuse
	Additions	Removed	Added +, Removed -		(48.0 kg)
	(liters)	(liters)	(liters)	(liters)	(liters)
1/1980	5.6	7.52	- 1.92	60.45	1.26
2/1980	11.2	9.39	+ 1.81	62.26	1.30
3/1980	16.8	15.35	+ 1.45	63.71	1.33
4/1980	16.8	17.42	- 0.62	63.09	1.31
5/1980	16.8	16.03	+ 0.77	63.86	1.33
6/1980	11.2	13.54	- 2.34	61.52	1.28
7/1980	5.6	7.58	- 1.98	59.54	1.24
8/1980	5.6	5.08	+ 0.52	60.06	1.25
9/1980	5.6	5.65	- 0.05	60.01	1.25
10/1980	11.2	11.49	- .29	59.72	1.24
11/1980	11.2	11.19	+ 0.01	59.73	1.24
12/1980	11.2	13.63	- 2.43	57.30	1.19
1/1981	5.6	7.41	- 1.81	55.49	1.16
2/1981	11.2	9.8	+ 1.4	56.89	1.19
3/1981	16.8	15.31	+ 1.49	58.38	1.22
4/1981	16.8	18.45	- 1.65	56.73	1.18
5/1981	16.8	15.93	+ 0.87	57.60	1.20
6/1981	11.2	14.98	- 3.78	53.82	1.12
7/1981	2.8	2.8	0.0	53.82	1.12

^aLysimeter UC-C contains Municipal Solid Waste.^bBoiled and cooled tap water was added weekly, and leachate was removed weekly.^cThe initial volume was obtained by subtracting the total dry weight from the total wet weight.

TABLE A-4. HYDROLOGIC RECORD OF LYSIMETER UC-D^a

Date (Month/ year)	Monthly Liquid Exchange in Lysimeter			Moisture Retained in Cell	
	Water ^b Additions (liters)	Leachate Removed (liters)	Net Added +, Removed - (liters)	Kg ^c (liters)	Kg H ₂ O/ Kg Dry Refuse (48.2 kg) (liters)
Initial	— ^d	—	—	24.4	0.51
8/1978	14.4	—	+ 14.4	38.8	0.80
9/1978	27.12	9.78	+ 17.34	56.14	1.16
10/1978	11.2	10.02	+ 1.18	57.32	1.19
11/1978	11.2	10.55	+ .65	57.97	1.20
12/1978	11.2	12.56	- 1.36	56.61	1.17
1/1979	5.6	5.65	- .05	56.56	1.17
2/1979	11.2	9.17	+ 2.02	58.58	1.22
3/1979	16.8	14.65	+ 2.15	60.73	1.26
4/1979	16.8	16.15	+ .65	61.38	1.27
5/1979	16.8	17.42	- .62	60.76	1.26
6/1979	11.2	11.67	- .47	60.29	1.25
7/1979	5.6	7.37	- 1.77	58.52	1.21
8/1979	5.6	6.32	- .72	57.80	1.20
9/1979	5.6	5.58	+ .02	57.82	1.20
10/1979	11.2	9.51	+ 1.69	59.51	1.23
11/1979	11.2	10.74	+ 0.46	59.97	1.24
12/1979	11.2	11.42	- 0.22	59.75	1.24
1/1980	5.6	7.28	- 1.68	58.07	1.20
2/1980	11.2	9.96	+ 1.24	59.31	1.23
3/1980	16.8	15.22	+ 1.58	60.89	1.26
4/1980	16.8	17.64	- 0.84	60.05	1.25
5/1980	16.8	16.08	+ 0.72	60.77	1.26
6/1980	11.2	13.42	- 2.22	58.55	1.21
7/1980	5.6	5.64	- 0.04	58.51	1.21

^aLysimeter UC-D contains Municipal Solid Waste.^bBoiled and cooled tap water was added weekly, and leachate was removed weekly.^cThe initial volume was obtained by subtracting the total dry weight from the total wet weight.^dNot determined.

TABLE A-5. HYDROLOGIC RECORD OF LYSIMETER UC-E^a (PART I)

Date (Month/ year)	Monthly Liquid Exchange in Lysimeter			Moisture Retained in Cell	
	Water ^b	Leachate	Net	Kg ^c	Kg H ₂ O/ Kg Dry Refuse
	Additions	Removed	Added +, Removed -	18.1	(38.7 kg)
	(liters)	(liters)	(liters)	(liters)	(liters)
Initial	-- ^d	—	—	18.10	0.47
8/1978	12.2	—	+ 12.2	30.30	0.78
9/1978	23.82	4.25	+ 19.57	49.87	1.29
10/1978	11.2	9.39	+ 1.81	51.68	1.34
11/1978	11.2	10.02	+ 1.18	52.86	1.37
12/1978	11.2	12.93	- 1.73	51.13	1.32
1/1979	5.6	5.9	- .3	50.83	1.31
2/1979	1.2	8.89	+ 2.31	53.14	1.37
3/1979	16.8	15.18	+ 1.62	54.76	1.41
4/1979	16.8	16.42	+ .38	55.14	1.42
5/1979	16.8	17.54	- .74	54.40	1.41
5/6/1979	11.2	11.71	- .51	53.89	1.39
7/1979	5.6	7.37	- 1.77	52.12	1.35
8/1979	5.6	6.31	- .71	51.41	1.33
9/1979	5.6	5.33	+ .27	51.68	1.34
10/1979	11.2	10.18	+ 1.02	52.70	1.36
11/1979	11.2	10.59	+ 0.61	53.31	1.38
12/1979	11.2	11.33	- 0.13	53.18	1.37

^aLysimeter UC-E contains Hospital Waste + Municipal Solid Waste.^bBoiled and cooled tap water was added weekly, and leachate was removed weekly.^cThe initial volume was obtained by subtracting the total dry weight from the total wet weight.^dNot determined.

* TABLE A-5. HYDROLOGIC RECORD OF LYSIMETER UC-E^a (PART II)

Date (Month/ year)	Monthly Liquid Exchange in Lysimeter			Moisture Retained in Cell	
	Water ^b	Leachate	Net	Kg ^c	Kg H ₂ O/ Kg Dry Refuse
	Additions	Removed	Added +, Removed -		(38.7 kg)
	(liters)	(liters)	(liters)	(liters)	(liters)
1/1980	5.6	7.64	- 2.04	51.14	1.32
2/1980	11.2	10.04	+ 1.16	52.30	1.35
3/1980	16.8	15.34	+ 1.46	53.76	1.39
4/1980	16.8	17.63	- 0.83	52.93	1.37
5/1980	16.8	16.04	+ 0.76	53.69	1.39
6/1980	11.2	12.71	- 1.51	52.18	1.35
7/1980	5.6	7.44	- 1.84	50.34	1.30
8/1980	5.6	5.42	+ 0.18	50.52	1.31
9/1980	5.6	5.64	- 0.04	50.48	1.30
10/1980	11.2	11.83	- 0.63	49.85	1.29
11/1980	11.2	10.42	+ 0.78	50.63	1.31
12/1980	11.2	12.92	- 1.72	49.81	1.29
1/1981	5.6	7.22	- 1.62	47.29	1.22
2/1981	11.2	9.57	+ 1.63	48.92	1.26
3/1981	16.8	15.28	+ 1.52	50.44	1.30
4/1981	16.8	17.62	- .82	49.62	1.28
5/1981	16.8	15.92	+ .88	50.50	1.30
6/1981	11.2	16.58	- 5.38	45.12	1.17
7/1981	11.2	11.21	+ .01	45.13	1.17

^aLysimeter UC-E contains Hospital Waste + Municipal Solid Waste.

^bBoiled and cooled tap water was added weekly, and leachate was removed weekly

^cThe initial volume was obtained by subtracting the total dry weight from the total wet weight.

TABLE A-6. HYDROLOGIC RECORD OF LYSIMETER UC-F^a

Date (Month/ year)	Monthly Liquid Exchange in Lysimeter			Moisture Retained in Cell	
	Water ^b Additions (liters)	Leachate Removed (liters)	Net Added +, Removed - (liters)	Kg ^c (liters)	Kg H ₂ O/ Kg Dry Refuse (34.5 kg) (liters)
Initial	— ^d	—	—	11.4	0.33
8/1978	12.2	—	12.2	23.6	0.68
8/1978	12.2	—	+ 12.2	35.8	1.01
9/1978	49.72	18.37	+ 31.35	54.95	1.59
10/1978	11.2	9.28	+ 1.92	56.37	1.63
11/1978	11.2	11.02	+ 0.18	56.55	1.64
12/1978	11.2	13.38	- 2.18	54.37	1.58
1/1979	5.6	5.06	+ 0.54	54.91	1.59
2/1979	11.2	9.2	+ 2.00	56.91	1.65
3/1979	16.8	14.93	+ 1.87	58.78	1.70
4/1979	16.8	16.58	+ 0.22	59.00	1.71
5/1979	16.8	17.38	- 0.58	58.42	1.69
6/1979	11.2	11.48	- 0.28	58.14	1.69
7/1979	5.6	7.34	- 1.74	56.40	1.63
8/1979	5.6	6.12	- 0.52	55.88	1.62
9/1/1979	5.6	5.18	+ 0.42	56.30	1.63
10/1979	11.2	9.88	+ 1.32	57.62	1.67
11/1979	11.2	10.21	+ 0.99	58.61	1.70
12/1979	11.2	11.24	- 0.04	58.57	1.70
1/1980	5.6	7.34	- 1.94	56.63	1.64
2/1980	11.2	9.92	+ 1.28	57.91	1.68
3/1980	16.8	15.41	+ 1.39	59.30	1.72
4/1980	16.8	17.58	- 0.78	58.52	1.70
5/1980	16.8	15.94	+ 0.86	59.38	1.72
6/1980	11.2	13.4	- 2.20	57.18	1.66
7/1980	5.6	7.77	- 2.17	55.01	1.59
8/1980	1.4	1.14	+ 0.26	55.27	1.60

^aLysimeter UC-F contains Hospital Waste.

^bBoiled and cooled tap water was added weekly, and leachate was removed weekly.

^cThe initial volume was obtained by subtracting the total dry weight from the total wet weight.

^dNot determined.



B. MEDIA AND REAGENTS

Media and Reagents

N-Acetyl-L-Cysteine-HaOH Solution

Vestal (1970)

To digest concentrated leachate preparations.

- | | | |
|------------------------------|-----|----|
| o 4% NaOH, sterile | 50 | ml |
| o 2.9% Sodium citrate | 50 | ml |
| o N-acetyl-L-Cysteine powder | 0.5 | g |

API-20A

Analytab Products, Inc., Carle Place, New York.

API-20E

Analytab Products, Inc., Plainview, New York 11803

Azide Dextrose broth (Difco Laboratories, Detroit Michigan)

Bacitracin Susceptibility Test

To separate Group A streptococci from other beta hemolytic streptococci.

- o Blood Agar plate containing 5% sheep-blood
- o Bacitracin Differential Disc (Difco) containing 0.04 units

Bile, 40% in Brain Heart Infusion Broth

- | | | |
|--|-----|----|
| o Brain heart infusion broth | 600 | ml |
| (BBL, a Division of Becton,
Dickerson & Co., Corkeys- | | |

ville, Maryland 21030), prepared

o 10% oxgall (Difco) solution 400 ml

Sterilized by membrane filtration.

Mix aseptically.

Bile Esculin Agar (Difco)

Bird Seed Agar

Rippon (1974)

o Thistle seeds	70.00 g
Creatinine	0.78 g
Chloranphenical	0.05 g
Glucose	10.00 g
Agar	20.00 g
Diphenyl	100 mg
Deionized distilled water	1 L

Pulverize seed, suspend in 350 ml of water and autoclave for 10 minutes at 121 C. Filter and bring volume to 1 liter. Dissolve all ingredients except diphenyl and sterilize at 121 C for 15 minutes. Cool to 45 C. Add diphenyl (dissolved in 10 ml of 95% ethanol). Mix and pour on plates. This medium differentiates Cryptococcus neoformans from other yeast agents by producing dark brown gelatinous colonies.

Bismuth Sulfite (Wilson and Blair) Medium (BBL)

Blood Agar

- | | | |
|---------------------------|----|---|
| o Blood Agar Base | 40 | g |
| Deionized Distilled Water | 1 | L |

Sterilized at 121 C for 15 minutes. Cool to 50 C and add 50 ml of sheep defibrinated blood from Flow Laboratories, McLean, Virginia 22102. For anaerobic bacteria incubate in a GasPak (BBL) jar.

Blood Agar, for Streptococci

- | | | |
|--|----|----|
| o Blood Agar base (heart infusion, Difco), prepared | 1 | L |
| o Sheep or horse defibrinated blood (Flow Laboratories). | 50 | ml |

Mix aseptically.

Brain Heart Infusion Agar (Difco)

Brain Heart Infusion Broth (BBL)

Brilliant Green Bile 20% (Difco)

Brown's Methane Utilization Medium

Brown et al. (1964)

To grow methane utilizing bacteria.

o KNO ₃	1.0 g
K ₂ HOP ₄ .3H ₂ O	.5 g
MgSO ₄ .7H ₂ O	.2 g
FeCL ₃ .6H ₂ O	.05 g
Deionized distilled water	1 L

Sterilize at 121 C for 15 minutes.

Cooked Meat Medium (Difco)

Chopped Meat Medium

Dowell and Hawkins (1976)

To initiate growth in stressed clostridia.

o Ground meat (fat free)	500 g
Deionized distilled water	1 L
1N NaOH	25 ml

Bring to a boil and stir. Cool and refrigerate overnight. Skim off fat. Filter through gauze. Spread out meat to dry.

To the liquid:

o Deionized distilled water	restore volume to 1 L
Peptone (Difco)	30 g
Yeast extract (Difco)	5 g

Potassium phosphate 5 g

Adjust pH to 7.8 with 1N NaOH

Dispense meat and liquid to tubes so that the proportion of meat to liquid is 1:4. Add a few grains of iron filings. Sterilize at 121 C for 15 minutes.

Corn Meal Agar

To differentiate yeast, to show chlamydospore production by Candida albicans.

o Yellow cornmeal (Quaker)	62.5 g
Bacto Agar (Difco)	19.0 g
Deionized distilled water	1 L

Heat cornmeal and water in a water bottle at 52°C for 1 hr. Filter. Dilute the filtrate to 1500 ml with deionized distilled water. Add the agar and autoclave at 121 C for 15 minutes.

Decarboxylase Base Moeller (Difco)

o Decarboxylase Medium Base (Difco	10.5 g
L-Lysine (Difco, Arginine	10.0 g
or L-Ornithine -HCl,	
(Difco) to produce a 1% solution	
Deionized distilled water	1 L

Sterilize at 121 C for 10 minutes. When ornithine is added, the medium needs to be adjusted so that a final pH after sterilization will be 6.0. This adjustment requires the addition of 4.6 ml of 10 N NaOH.

Deoxyribonuclease Agar (Difco)

Test Reagent

- o 1N HCl - clearing around colonial growth indicates a positive test

10% Diatomaceous Earth Mixture

- | | | |
|---------------------------------|-----|----|
| o Diatomaceous Earth | 100 | g |
| (Celite 545, Fisher Scientific | | |
| Co., Fairlawn, New Jersey 07410 | | |
| Deionized distilled water | 900 | ml |

Sterilized at 121 C for 15 minutes.

Dulcitol-Selenite Broth

Bordner et al. (1978)

An enrichment broth for Salmonella.

- | | | |
|-----------------------------|------|---|
| o Proteose Peptone | 4.0 | g |
| Yeast extract | 1.5 | g |
| Dulcitol | 4.0 | g |
| Sodium Selenite | 5.0 | g |
| Disodium hydrogen phosphate | 1.25 | g |

Potassium dihydrogen phosphate	1.25 g
Deionized distilled water	1 L
Final pH:	6.9

Heat to dissolve. Do not boil and do not autoclave.

EC Medium (Difco)

EDTA (disodium ethylene diamine tetraacetate)

- o Using a formula weight of 372 g, EDTA (disodium ethylene diamine tetraacetate, Fisher Scientific Co., Fairlawn, New Jersey 07410) in weights of 3.72 g and 0.37 g, were added to separate 100 ml glass bottles and were sterilized so that when 100 ml of leachate was added the final concentrations of EDTA would be: 0.1 M; and 0.01 M, respectively. The glass bottles containing these amounts of EDTA were sterilized at 121 C for 15 minutes previous to use.

Egg Yolk Agar

- o McClung and Turbe egg yolk agar 900 ml (Difco) prepared with deionized distilled water sterilized and cooled to 50 C

- o Sterile egg yolks, prepared by 100 ml
adding an equal volume of egg yolk
and sterile saline solution.
Mix aseptically.

m-Enterococcus Agar (Difco)

Eosin Methylene Blue Agar (Difco)

Esculin Broth

- o Heart infusion broth (Difco) 25 g
- Esculin 1 g
- Agar (Difco) 1 g
- Deionized distilled water 1 L
- Final pH = 7.0

Sterilized at 121 C for 15 minutes.

Ethyl Violet Azide broth (Difco)

Fermentation Test Medium (Clostridium)

Dowell and Hawkins (1970)

- o Sterilized CHO medium (Difco) 1 L
prepared with deionized
distilled water

c 10% aqueous solution of 100 ml
lactose sterilized by membrane
filtration. Mix aseptically with
CHO medium.

o 1% Brom thymol blue solution 10 ml

Fermentation Test Medium (Gram-negative Rods)

o Phenol Red Broth Base (BBL) 1 L
with fermentation vials prepared
and sterilized at 118 C for 15 minutes

o 10% Lactose, maltose, or sucrose 100 ml

Aqueous solutions sterilized by membrane filtrations.

Fermentation Test Medium (Gram-Positive Non-sporeforming
Rods)

o Purple broth base (BBL), prepared 1 L
and sterilized

o 10% sugar solution of lactose, 100 ml
sucrose, and maltose membrane
filter sterilized.

The two solutions were aseptically mixed and divided into
tubes.

Fermentation Test Medium (Streptococci)

- o Purple Broth Base (BBL), 1 L prepared, sterilized broth.
- o 5 ml of glycerol is added to the purple broth base prior to autoclaving. Also, add 5 g of yeast extract (Difco) per liter of broth. This is a necessary ingredient for glycerol fermentation only.
- o 10% solution of lactose, 100 ml arabinose, or sorbitol was sterilized by membrane filtration.

Gases - Natural Gas

To grow methane utilizing bacteria.

- o Natural gas, methane, 94% pure (Rickabaugh, 1982, personal communication)

Gases - Nitrogen

To flush out oxygen and maintain an anaerobic condition.

- o Nitrogen gas, 99.0% pure, Puritan-Bennett, Cincinnati, Ohio.
- o Nitrogen Gas, CGA-580, 99.99% pure, oxygen free, Union Carbide Corporation, Linde Division, Birmingham, Alabama.

Gases - Pure

To grow methane utilizing and methane producing bacteria.

- o Hydrogen, mole percentage 99.95% #3-00-100
- o Carbon dioxide, mole percentage 99.80% #2-3402
- o Methane, mole percentage 99.00%, #2-2562

Supelco, Inc., Bellefonte, Pennsylvania 16823

GN Broth (Hajna) BBL

Enrichment broth for Salmonella and Shigella.

Gram Stain

- o Burke and Kopeloff - Beerman Modification
(Society of American Bacteriologists, 1957).

Hektoen Enteric Agar (Difco)

Horse Serum

Flow Laboratories

For germ tube test (Sylvé-Hutner and Cooper, 1980)

Hydrogen Peroxide (3%)

Inhibitory Mold Agar (BBL)

To grow fungal pathogens, especially the deepseated fungi.

Iron Milk

Dowell and Hawkins (1976)

o Skim milk powder (BBL)	100	g
Deionized distilled water	1	L

Sterilize at 115 C for 20 minutes.

Karung's Medium

Karung and yegim (1954)

To aid in demonstrating dimorphism of Blastomyces,
Histoplasma, and Sporothrix.

o Potato flour	1	g
Distilled water	100	ml

Heat until the flour congeals. Sterilize at 121 C for 15 minutes. Cool. Add egg to the mixture. Tube and inspissate in a slanting position for 1 hour at 90 C.

Kligler Iron Agar (Difco)

Lactophenol Cotton Blue Stain

Conant et al. (1971)

To stain the mold hyphae.

o Phenol crystals	20	g
Lactic acid	20	ml
Glycerol	40	ml
Distilled water	20	ml

Dissolve under hot tap water. Add 0.05 g cotton blue.

Lauryl Sulfate Broth (BBL)

Leadbetter and Foster Medium

Leadbetter and Foster (1958)

For the enrichment and isolation of methane-utilizing bacteria.

o NaNO_3	0.2 g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.02 g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.1 mg
Na_2HPO_4	0.02 g
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	9.0 mg
Cu (as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)	0.5 ug
B (as H_3BO_3)	1.0 ug
Zn (as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$)	7.0 ug
Mo (As MoO_3)	1.0 ug
KCl	4.0 mg
CaCl_2	1.5 mg
Water	100 ml

Final pH: 7.0

Sterilize at 121°C for 15 minutes.

Litmus Milk (Difco)

Lowenstein, Medium, Jensen (Difco)

Lugol's Iodine

Society of American Bacteriologists (1957)

For starch hydrolysis.

Lysine Iron Agar (BBL)

MacConkey Agar (Difco)

0.1% Methylene Blue in Skim Milk

Bordner et al. (1978)

o Skim Milk Powder (BBL)	100	g
Methylene Blue Powder	1	g
Distilled Water	1	L

Heat to dissolve. Sterilize at 115°C for 20 minutes.

Methyl Red - Voges Proskauer Medium (Difco)

Standard Methods (1976)

Voges-Proskauer Test Reagents

Standard Methods (1976)

- o Solution #1
5% L-naphthol in 95% ethyl alcohol
- o Solution #2
40% aqueous potassium hydroxide

Methyl Red Reagent

o Methyl red inidcator	0.1	g
95% ethanol	300	ml
Deionized distilled water	200	ml

Dissolve the methyl red with ethyl alcohol and dilute to 500 ml.

Methanogenesis Medium

(Paynter and Hurgate, 1968; Zerkus and Winfrey, 1970; and Strayer and Tiedje, 1978)

Enrichment broth for methane-producing bacteria.

o Sewage Sludge	300	ml
KH ₂ PO ₄	0.5	g
K ₂ HPO ₄	0.5	g
NaCl	1.0	g
(NH ₄) ₂ SO ₄	0.5	g
MgSO ₄ ·7H ₂ O	6.2	g
CaCl ₂ ·2H ₂ O	0.13	g
Reasurin	0.001	g
MnSO ₄ ·H ₂ O	0.5	g
FeSO ₄ ·7H ₂ O	0.2	g
CoCl ₂ ·6H ₂ O	0.17	g
ZnSO ₄ ·7H ₂ O	0.18	g
CuSO ₄ ·5H ₂ O	0.02	g

Na ₂ SO ₄ ·12H ₂ O	0.018 g
H ₃ BO ₄ (Boris Acid)	0.01 g
Na ₂ MoO ₄ ·2H ₂ O	0.01 g
Trypticase (or tryptone)	2.0 g
Yeast Extract	2.0 g
Na Formate	2.0 g
Na Acetate	2.0 g
Water	700 ml

Place 100 ml of medium in 150 ml glass vials and flush with oxygen free nitrogen. Sterilize at 121 C for 15 minutes. Re-flush with oxygen free nitrogen and secure mouth of vials with serum sleeve rubber stoppers. Cool.

- o 50% NaHCO₃ 0.25 ml
- o Escherichia coli ATCC 13706, 0.50 ml
overnight culture grown at 37 C in brain
heart infusion broth.

Middlebrook 7H9 Agar

To store Mycobacterium isolates.

- o Dehydrated Middlebrook 7H9 4.7 g
broth base
- Agar (BBL) 15. g
- Malachite Green .001 g
- Deionized distilled water 1 L
- o Middlebrook ADC Enrichment (BBL) 100 ml

Middlebrook 7H9 Broth

To grow mycobacteria inocula for susceptibility tests.

- o Middlebrook 7H9 broth base (BBL) 900 ml
cool to 45°C
- o Middlebrook ADC Enrichment (BBL) 100 ml

Middlebrook 7H10 Agar

To enumerate mycobacteria.

- o Middlebrook and Cohn 7H10 900 ml
Agar Base (BBL)
- o Middlebrook OADC Enrichment (BBL) 100 ml

Middlebrook 7H10 Agar with Antibiotics

For Mycobacterium antibiotics susceptibility test.

- o Middlebrook and Cohn 7H10 900 ml
Agar Base (BBL)
- o Middlebrook OADC Enrichment (BBL) 100 ml

Mix. Pour into plastic petri dishes divided into quadrants (Curtin-Matheson Scientific Co., Cincinnati, Ohio 45246, Quad-Petri sterilized disposable polystyrene). Dispense 5.0 ml of medium into each quadrant.

- o Antibiotic discs:
 - Isoniazid (BBL), 1 and 5 mcg
 - Ethanbutol (BBL), 25 and 50 mcg
 - P-aminosalicylic acid (BBL), 10 and 50 mcg

- Rifampin (BBL), 5 and 25 mcg
- Streptomycin (BBL), 10 and 50 mcg

These were added to 10 quadrants on three plates,
leaving two quadrants without antibiotics for controls.

Milk, Skim, Powder (BBL)

Mineral Oil

Society of American Bacteriologists (1957)

Sterilize in 100 ml amounts at 121 C for 60 minutes.

MIO Medium (Difco)

Motility Test Medium

- o Motility Test Medium (BBL) 22 g
- o Deionized distilled water 1 L

Cooled to 50°C following sterilization at 121 C for 15
minutes. Add TTC solution.

- o 1% TTC Solution, prepared with Bacto-TTC 5 ml
(2,3,5-Triphenyl tetrazolium chloride and deion-
ized distilled water) and membrane filter
sterilized before use.

Mycosel Agar (BBL)

To isolate the dermatophytes.

Nagler Test Reagents

Koneman et al. (1979)

To type clostridial isolates with Clostridium
perfringens (welchii) type A and type B antisera:

- o Clostridium welchii, type A antiserum, Rx 6753
- o Clostridium welchii, type B antiserum, Rx 3243

From Wellcome-Burroughs, Animal Health Division, Kansas
City, Missouri.

For the typing test, the antisera were added to egg yolk
plate by adding two drops to one-half of an egg yolk
agar plate. The antiserum was smeared over the agar
surface with a sterile bacterial transfer wire loop.
The antisera usually absorbed in the agar within 30
minutes.

Nitrate Broth (Difco)

Nitrate Test Reagents

Finegold et al. (1978)

- o Solution A:

Sulfanilic Acid	8	g
Acetic Acid, 5 N	1	L
- o Solution B:

L-naphthylamine	5	g
Acetic Acid, 5 N	1	L
- o Zinc dust

Nutrient Gelatin (BBL)

Oxidase Test Reagents

N,N,N¹,N¹-tetramethyl-p-phenylenediamine dihydrochloride, 1% solution in droppers. Manufactured by Marion Scientific Corporation, Kansas City, Missouri 64114.

Oxidation-Fermentation Test Medium

- o OF Basal Medium (Difco) 900 ml
 - o 10% glucose aqueous solution, 100 ml
- sterilized by membrane filtration.

Mix aseptically. After inoculation overlay one tube with sterile mineral oil.

1% Peptone Water

Duncan and Harmon (1976)

A diluent for anaerobic bacterial cultures

- o Bacto-peptone (Difco) 10 g
- Deionized distilled water 1 L

Sterilized at 121°C for 15 minutes.

pH 9.6, Brain Heart Infusion Broth

Bordner et al. (1978)

- o Brain Heart Infusion Broth (BBL) 1 L
- with the addition of enough
Na₃PO₄·12H₂O to produce a final pH of 9.6.

Phenylalanine Agar (Difco)

Phenylethanol Agar (Difco)

Phosphate Buffer

Standard Methods (1976)

Phosphate Buffer, pH 6.8

Vestal (1976)

To neutralize the N-acetyl-L-Lysteine NaOH solution at the end of the digestion period.

- o Na_2HPO_4 , anhydrous 9.47 g/l of 500 ml
deionized distilled water, sterile .
- o KH_2PO_4 , 9.07 g/l of deionized 500 ml
distilled water.

Mix, sterilize at 121°C for 15 minutes.

Pseudusel Agar (BBL)

Sabouraud Agar, Modified (Difco)

Saline

- o Sodium chloride 8.5 g
- deionized distilled water 1 L

Sterilized at 121°C for 15 minutes.

Salmonella Antisera (Difco)

- o Salmonella O Antiserum Poly A (Difco)
- o Salmonella O Antiserum Poly B (Difco)
- o Salmonella Vi Antiserum (Difco)

Selenite F Broth (BBL)

SIM (Difco)

Simmons Citrate Agar (BBL)

6.5% Sodium Chloride in Brain Heart Infusion Broth

Bordner et al. (1978)

- | | | |
|------------------------------------|----|---|
| o Brain Heart Infusion Broth (BBL) | 1 | L |
| prepared | | |
| o Sodium chloride | 65 | g |

Mix and autoclave at 121 C for 15 minutes.

Starch Agar

Bordner et al. (1978)

- | | | |
|---------------------------|----|---|
| o Peptone (Difco) | 10 | g |
| Yeast enrichment (Difco) | 5 | g |
| Sodium Chloride | 5 | g |
| Starch (Soluble) | 2 | g |
| Agar (Difco) | 15 | g |
| Deionized distilled water | 1 | L |

Heat to dissolve. Sterilized at 121 C for 15 minutes.

Standard Methods Agar (BBL)

Streptex Streptococcal Grouping Antisera

To place isolated strains of streptococci into
Lancefield serological groups A, B, C, D, E, F, or G.
Manufactured by Wellcome Reagents Limited, Welcome
Research Laboratories, Beckenham, England 13R3 3BS.

Sodium Hippurate Broth

Facklam et al. (1974)

To identify Group B Streptococci.

o Heart Infusion Broth (Difco)	25.0	g
Sodium hippurate	10.0	g
Distilled water	1	L

Sterilize at 121°C for 15 minutes.

o Test Solution:

FeCl ₃ .6H ₂ O	12	g
HCl, 21% prepared by 5.4 ml of concentrated HCl to 94.6 ml of deionized distilled water.	100	ml

SPS Agar (Sulfite Polymyxin Sulfadiazine Agar [BBL])

Angelotti, et al. (1962)

0.04% Tellurite Agar

Bordner et al. (1978)

For plate counts of non-spore-forming Gram-positive rods.

- o Brain heart infusion broth (BBL) 1 L
prepared and sterilized and cooled
to 50 C.
- o 4% potassium tellurite, 10 ml
sterilized by membrane filtration.

Mix. This gives a final potassium tellurite concentration of 0.04%.

Tetrathionate Broth

- o Tetrathionate Broth Base (Difco) 1 L
prepared with deionized distilled
water, boiled and cooled to 60 C.
- o Iodine Solution prepared by 20 ml
adding
Iodine 6 g
Potassium iodide 5 g
Deionized distilled water 20 ml

Add iodine solution to broth.

Thiogel Medium (BBL)

Thioglycollate Medium without Indicator - 135°C (Difco)

Triple Sugar Iron Agar (BBL)

Trypticase Soy Agar (BBL)

Tryptone Sulfite Cycloserine (TSC) Agar

Duncan and Harmon (1976)

To enumerate Clostridium perfringens

o Trypticase Soy Broth (BBL)	30.0 g
Sodium Metabisulfite	1.83 g
Ferric Ammonium Citrate	1.0 g
Agar	20.0 g
Yeast Extract	5.0 g
Distilled Water	1 L

Heat to dissolve ingredients. Adjust pH to 7.6.

Sterilize at 121 C for 10 minutes. Cool to 50 C. To each liter of medium add 10 ml of 4.0% D-cycloserine (sterilized by the membrane filter) and 80 ml of sterile 50% egg yolk in saline.

Tryptophane Broth (BBL)

To determine indol production.

o Trypticase peptone (BBL)	15 g
Deionized distilled water	1 L

Sterilize at 121 C for 15 minutes.

o Test Reagent (Koovac's Reagent)

Isoamyl alcohol	150	ml
p-dimethyl-amino benzaldehyde	10	g
HCl concentrated	50	ml

Dissolve the aldehyde in the alcohol in a 50 to 60°C water bath. Carefully add the acid to the cooled mixture.

TTC Agar

Bordner et al. (1978)

For tetrazatium hydrolysis.

o Peptone (Difco)	10	g
Beef extract	10	g
Sodium Chloride	5	g
Agar	14	g
Deionized distilled water	1	L
Final pH: 7.0 - 7.3		

Autoclave at 121 C for 15 minutes.

- | | | |
|---|----|----|
| o 1% TTC Solution (2,3,5-triphenyl tetrazalium chloride) filter sterilized. | 10 | ml |
| o 50% glucose solution sterilized by membrane filter. | 20 | ml |

Urea Agar (Christenson's)

- o Urea Agar Base (Difco) 29 g
- Deionized distilled water 100 ml

Sterilize by the membrane filtration. Add agar.

- o Agar (Difco), 15 g in 900 ml 900 ml
- of water, and sterilized at 121 C for
- 15 minutes, and cooled to 50 C. Mix, tube and
- slant (Washington, 1981).

Ziehl-Neelson Stain

Vestal (1976); Runyon et al. (1980)

Acid-fast stain for Mycobacterium-like colony
preparations.

C. TEST PROCEDURES

TEST PROCEDURES

° Acid-Fast Stain

(Vestal, 1976; Koneman et al., 1979)

° Antibiotic Susceptibility Test for Mycobacteria

(Vestal, 1976)

Test Conditions:

Mycobacterial inoculum (0.1 ml of a 10^{-1} to 10^{-4} dilution in saline) made from a 7 day old culture in Middlebrook 7H9 broth incubated at 37C, was placed on a prepared Middlebrook 7H10 agar plate containing an antibiotic disc within the agar. The plates were incubated at 37C for 24 hr right side up and were inverted and incubated for three weeks in a plastic bag.

Test Results:

Inhibition of Mycobacterial growth.

° Antibiotic (bacitracin) Susceptibility Test for Streptococci

(Facklam et al., 1974)

Test Conditions:

Culture was streaked on blood plate. Then a bacitracin differential disc was placed on the agar surface. The plate was incubated at 37C for 24 hr.

Test Results:

A zone of growth inhibition around the disc indicated a positive test and the presence of Group A Streptococci.

• API 20A, and API 20E

(Analytab Products, Inc.)

• 40% Bile in Brain Heart Infusion Broth, Growth

(Bordner et al., 1978)

• Bird Seed Agar

(Silva Hutner & Cooper, 1980)

Test Conditions:

Incubated bird seed agar plates for 1 week at 30C.

Test Results:

Cryptococcus neoformans produced brown colonies, a property not shown by other yeasts.

• Capsules, Cryptococcus Neoformans

(Finegold, et al., 1978; Silva-Hutner & Cooper, 1980)

Test Conditions:

Placed culture and India ink on slide. Added a cover slip and examined microscopically.

Test Results:

Cryptococcus neoformans cultures showed round budding cells with capsules.

• Catalase Test

(Bordner et al., 1978)

• Chlamydospores, Candida albicans

(Koneman et al., 1978)

Test Conditions:

Grew Candida-like colonies on cornmeal agar at room temperature for 48 hr. 417

Test Results:

Candida albicans produced chlamydospores.

° Citrate Test

(Bordner et al., 1978)

° Decarboxylase Tests for Lysine and Ornithine, and
Dihydrolase Test for Arginine

(Bordner et al., 1978; MacFaddin, 1976)

Test Conditions:

Inoculated freshly prepared (or heated in boiling water for 10 minutes and cooled) decarboxylase test medium containing lysine, a second containing ornithine and a third containing arginine. Overlayed each tube with 0.5 cm of sterile mineral oil. Incubated at 37C for 4 days.

Test Results:

A positive result showed a change from straw-colored media to a rose-red color, indicating the presence of lysine, ornithine decarboxylases and
• arginine dihydrolase.

° Decarboxylase test, Lysine Iron agar

(Bordner et al., 1978)

° Deoxyribonuclease (D-Nase) Test

Test Conditions:

Inoculated growth in a single line across the agar. Incubated plate at 37C for 24 hr.

Test Results:

Flooded plate with 1 N HCl. A zone of clearing around the line of growth indicated a positive test.

° Test for Dimorphic Molds

(Schwartz, Jan, 1979. Personal communication; Karung and Yegim, 1954)

Test Conditions:

Inoculated Karung's medium and incubated at 37C up to two weeks.

Test Results:

Mold-like colonies converted to yeast form.

° Esculin Hydrolysis, Clostridium

(Washington, 1981)

Test Conditions:

Incubated inoculated Esculin Broth in a Gas Pak (BBL) jar at 37C for 48 hr.

Test Results:

Examined in an ultraviolet light. The presence of fluorescence indicated esculin hydrolysis.

° Esculin Hydrolysis, Streptococci

(Washington, 1981)

Test Conditions:

Inoculated a single line on the surface of the agar. Incubated at 37C for 24-48 hr.

Test Results:

Esculin hydrolysis is indicated by a zone of

blackening around the colonial growth.

° Fermentation Test, Phenol Red Broth

Bordner et al. (1978)

For Gram-negative rods

° Fermentation Test, Purple Broth Base

(Bordner et al., 1978)

For streptococci and Corynebacterium

° Fermentation Test, Triple Sugar Iron Agar

(Bordner et al., 1978)

° Gelatin Hydrolysis

(MacFaddin, 1978; Bordner et al., 1978; Washington, 1981)

Test Conditions:

Clostridium incubated at 37C for 48 hr.

Corynebacterium, Listeria, Gram-negative rods and
Streptococcus, incubated at 37C for 14 days.

Test Results:

Following incubation placed tubes at 5C for 2 hr.

Liquid medium indicated hydrolysis.

° Germ Tube Test for Candida albicans

(Silva-Hutner and Cooper, 1980)

Test Conditions:

Inoculated 0.5 ml of sterile horse serum with a
yeast culture (grown at 37C for 48 hr). Incubated at
37C for 2 to 4 hr. Examined microscopically.

Test Results:

Candida albicans will produce a germ tube extending

from the yeast cell.

° Gram Stain

(Standard Methods, 1976; Society for American Bacteriologists, 1957)

° Hemolysis

(Koneman et al., 1979; Cowan, 1974; Bordner et al., 1978)

Test Conditions:

Clostridium perfringens, inoculated blood agar plate incubated in a Gas Pak (BBL) at 37C for 48 hr. Corynebacterium and Streptococcus blood agar plates incubated at 37C for 48 hr.

Test Results:

Clostridium perfringens produced target zone hemolysis which is a double zone 1 to 2 cm in diameter. Corynebacterium-like Gram-positive rods produced hemolysis similar to streptococci. For streptococci, see Bordner et al. (1978).

° Indol Test

(Standard Methods, 1976)

° Lecithinase Production

(Koneman et al., 1979)

Test Conditions:

Incubated egg yolk plates in a Gas Pak (BBL) jar at 37C for 24 hr.

Test Results:

The lecithinase reaction, a large yellow zone of

precipitation, extends from the Clostridium perfringens colony 0.5 to 1 cm.

° Lipase Production

(Koneman et al., 1979)

Test Conditions:

Same as for lecithinase production.

Test Results:

A shining, iridescent reaction on the surface of the egg yolk plate. The diameter is 0.2 to 0.5 cm.

° MacConky Agar, Growth

(Koneman et al., 1979; Analytab Products, Inc.)

Test Conditions:

Incubated MacConky agar plates at 37C for 24 to 48 hr.

Test Results:

Growth.

° Methane Production

Gas chromatograph

° 0.1% Methylene Blue Milk

(Bordner et al., 1979)

Test Conditions:

Incubated tube of medium at 37C for 7 days. Read results daily.

Test Results:

Tube of media reduces from blue to white. Tube may reoxidize and give a false negative reaction unless

results are recorded daily.

° Methyl Red Test

(Standard Methods, 1976)

° Motility Test

(Bordner et al., 1978)

° Nagler Reaction

(Koneman et al., 1979)

Test Conditions:

Streaked culture on egg yolk plate, one-half with adsorbed antisera and the other side left untouched as a control.

Test Results:

When the antisera reacted with the clostridial toxins, the lecithinase production was inhibited. The control shows lecithinase production and the side containing the antisera shows no lecithinase production.

° Nitrate Reduction

(Bordner et al., 1978)

° Oxidase Reaction

(Bordner et al., 1978)

° Oxidation-Fermentation Test

(Cowan, 1974; Fineburg et al., 1978)

For Corynebacterium-like Gram-positive rods, and Gram-negative rods.

Test Conditions:

Inoculated two tubes (heated in boiling water for
423

10 minutes and cooled). Overlayed one tube with mineral oil to a height of 0.5 cm. Incubated at 37C for 5 days.

Test Results:

The fermentation reaction produced acid (tube turns from green to yellow) in the anaerobic tube.

The oxidation reaction produced acid in both the aerobic and the anaerobic tube.

The third alternative, a negative reaction, had no reaction in either tube.

pH 9.6 in Brain Heart Infusion Broth

For Streptococci

(Bordner et al., 1978)

° Phenylalanine Deaminase Test

(Bordner et al., 1978)

Test Conditions:

Incubated inoculated plate at 37C for 7 days.

Test Results:

The appearance of black colonial growth indicated tellurite hydrolysis reaction.

° Salmonella Antisera Type A, Type B, and Vi Antisera

(Bordner et al., 1978)

° 6.5% Sodium chloride, Growth

(Bordner et al., 1978)

° For Streptococci

Sodium Hippurate Hydrolysis Test

(Facklam et al., 1974)

Test Conditions:

Centrifuged a 48 hr. hippurate broth culture.
Placed 0.8 ml of the supernatant to a clean tube. Added
0.2 ml of ferric chloride reagent.

Test Results:

If a heavy precipitate remained longer than 10
minutes then the test is positive.

° Spore Production

(Finegold et al., 1978)

Test Conditions:

First method, heated an inoculated 0.1 ml in
distilled water in saline to 80C for 10 minutes.
Subculture to brain heart infusion broth and incubated
at 37C for 1 week to test for growth.

Gram stain preparations and looked for spores.

Second method, inoculated SIM medium. Incubated at
37C for 48 hr and then inoculated at room temperature
for 5 days. Gram stain at the end of a week and looked
for spores.

Test Results:

Found spores following microscopic examination of
Gram stain preparation.

° Starch Hydrolysis

(Bordner et al., 1978)

° Streptex Streptococcus Grouping Reactions

(Welcome Reagents, Ltd., Beckingham, England)

° Stormy Fermentation

(Koneman et al., 1979; Dowell & Hawkins, 1976)

Test Conditions:

Inoculated tube of iron milk, previously heated in boiling water for 10 minutes and cooled, and incubated in a Gas Pak (BBL) jar at 37C for 48 hr or up to a week following the initial inoculation.

Test Results:

Tube has milk digestion, coagulation, and gas production including multiple gas tracks around the sides of the tube.

° Temperature, Growth at 10C

(Borner et al., 1978)

Test Conditions:

Inoculated a 24 hr culture of Streptococcus in heart infusion broth by adding one drop to a tube of brain heart infusion broth. Incubated the tube at 10C for 5 days.

Test Results:

Growth.

° Temperature, Growth at 45C

(Bordner et al., 1978)

Test Conditions:

From a 24 hr culture (37C) of Streptococcus in heart infusion broth inoculated one drop into a tube of brain heart infusion broth. Incubated at 37C for 48 hr.

Test Results:

Growth.

- ° TTC Agar (2,3,5-Triphenyl Tetrazolium chloride)
(Bordner et al., 1978)

Test Conditions:

Inoculated plate and incubated at 37C for 48 hr.

Test Results:

The appearance of pink colonies indicated tetrazolium reduction.

- ° Urease Hydrolysis
(Bordner et al., 1979; Washington, 1981)
- ° Voges-Proskauer Test
(Bordner et al., 1978, Standard Methods, 1976)

D. THE MEASUREMENT OF METHANE-UTILIZING AND METHANE-
PRODUCING BACTERIA IN SOLID WASTE FROM THE
BOONE COUNTY LANDFILL

Appendix D

THE MEASUREMENT OF METHANE-UTILIZING AND METHANE-PRODUCING BACTERIA IN SOLID WASTE FROM THE BOONE COUNTY LANDFILL

INTRODUCTION

The purpose of this experiment was to determine the presence of methane utilizing bacteria in topsoil and the presence of methane-producing bacteria in landfill waste buried below. The use of soil cover over a landfill has many purposes, one of which is the control of decomposition gases such as methane. For this reason, measuring the concentration of methane-utilizing bacteria present in the topsoil was to determine if methane escaping the landfill had any selective value for increasing the numbers of these bacteria. Another objective was to determine if there was a difference in the concentration of methane-producing bacteria in the solid waste at three different levels within the landfill, namely, 5, 7, and 8-1/2 feet below the soil surface. This information would be useful in assessing the extent and stage of waste decomposition. As the means to carry out these assays were not available, procedures had to be developed. Therefore, the purpose of this work was two-fold: first, to test several available procedures which could determine the concentration of methane-using bacteria

and methane-producing bacteria from environmental samples; and second, to use these procedures to determine, respectively, the above biochemical activities on landfill topsoil covering and on solid waste samples taken at several levels within the landfill.

Methods of studying bacterial methane utilization and methane production can involve the use of radioisotopes. Simpler methods described in the literature, however, were used with the idea that methane and other gas concentrations could be measured by gas chromatographic techniques.

Accordingly, two experiments were run. One was to test the devised procedures using sewage sludge and soil from a wooded area as a positive sample, because both samples were thought to contain methane-users and methane-producers. The second experiment was to use these procedures to determine the presence of bacteria in the soil cover over the landfill (at 1.5 feet [0.46 meter]) which would use methane, and to determine the methane-producing bacteria in solid waste taken from the landfill depths of 5, 7, and 8.5 feet (1.5, 2.13, and 2.59 meters) below the surface. A control soil, taken from an area adjacent to the landfill, was used for comparisons. Soil was taken from this area at the 1.5, 5, 7, and 8.5 feet (0.46, 1.5, 2.13, and 2.59 meters) below the surface.

TEST PROCEDURES

Positive Samples Used to Test Procedures:

It was necessary to test the applicability of the test procedures by using positive samples. One positive sample, containing methane-utilizing bacteria, was present in soil taken from a moist humus area in a suburban woods in Cincinnati Ohio. Two kg samples were taken at the 1-1/2 foot (0.46 meters) level below the soil surface. This sample was taken in mid-winter. Therefore no icing of the soil was necessary for the trip back to the laboratory. This soil was kept at 5 C and used within eight hours. The other positive sample containing methane-producing bacteria, was sewage sludge (McCarty, 1964). This sewage sludge was obtained from the Miami Valley Treatment Plant in Cincinnati, Ohio on November 3, 1980. It was used both to test the selected test procedures as stated above and to serve as a nutrient in the methanogenesis medium. The soil from the woods described above also was used as a source of methane producing-bacteria, and was used to test the methane-producing procedures.

Landfill Sample Procedures:

The sampling site was the Boone County landfill, built by the EPA in 1971 (Chapter 3). The soil covering the landfill was taken at 1-1/2 feet (0.46 meter) below the

surface, and the solid waste was taken at levels of 5, 7, and 8-1/2 feet (1.5, 2.13, and 2.59 meter) below the soil surface. Control soil samples were dug at the same time. (See Figures 16 and 17) This control site was some 200 feet (60.96 meter) away from the landfill. These samples were obtained from the same depths as those from the landfill. All samples were dug with a backhoe within a five hour period. As each sample was uncovered, it was placed in a plastic bag and packed into a GasPak jar (BBL). These jars were kept in an ice chest during the trip back to the laboratory. There the jars were kept in a 20 C incubator for three months, at which time the final assays were made.

Treatment of Sample Prior to Microbial Enumeration

All solid waste samples were homogenized in the Waring blender prior to microbial enumeration and chemical analysis (Peterson, 1973). Twenty grams of the solid waste were placed in the Waring blender jar together with 180 ml of sterile distilled water or sterile phosphate buffer. The former was used to prepare samples for the chemical tests, as the pH nor the conductance could be determined in the presence of buffer. The Waring blender jar with the sample and the diluent was always placed in the biological safety hood to eliminate any possible microbial pollution by the spray from the blender during its operation. When anaerobes

were the object of the microbial enumeration, nitrogen gas was added to the blender to decrease the oxygen contact with the blended waste. The blender was routinely run at this high speed for 15 seconds. Following this the liquid portion of the blended material was used for the microbial and chemical analyses, as described in Standard Methods (1976) (See Figure D-1).

Growing Media:

Initially, two enrichment media (See Table D-1) were used to grow the methane-utilizing bacteria. Later, one was selected for further use. These were Brown's medium (Brown et al., 1964), and Leadbetter's medium (Leadbetter and Foster, 1958). The components of these broths are shown in Appendix B. Fifty milliliters of the liquid media were placed in 150 ml glass vials, stoppered with serum sleeves. The glass vials were sterilized at 121 C for 15 minutes. During the cooling process sterile serum sleeves were placed in the mouth of each vial. (See Figure D-2)

The methanogenesis medium (See Table D-1 and Appendix B) was adapted from those media described by Paynter and Humgate (1968), Zeikus and Winfrey (1976), and by Strayer and Tiedje (1978). Sewage sludge was used in place of the rumen fluid (Pfeffer, 1974). The medium was put in glass vials as described above, and sterilized for the same time

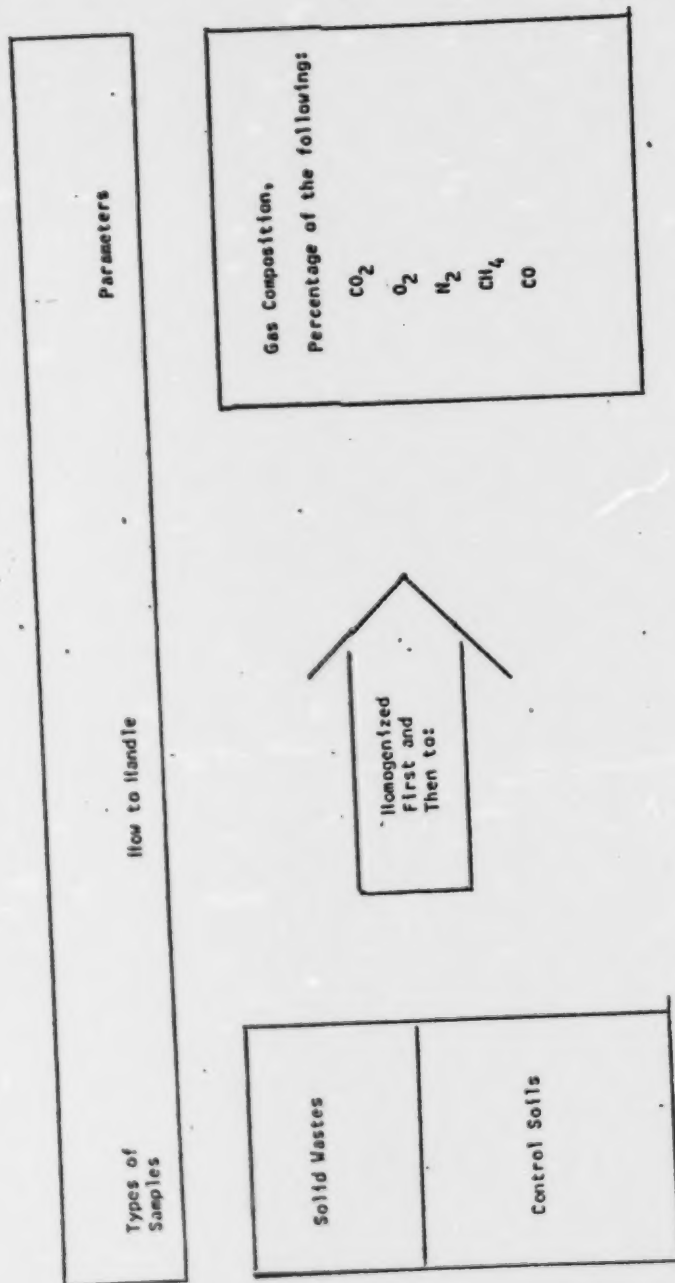


FIGURE D-1 PROCEDURES USED IN THE ANALYSIS OF SOLID WASTE AND LEACHATE SAMPLES

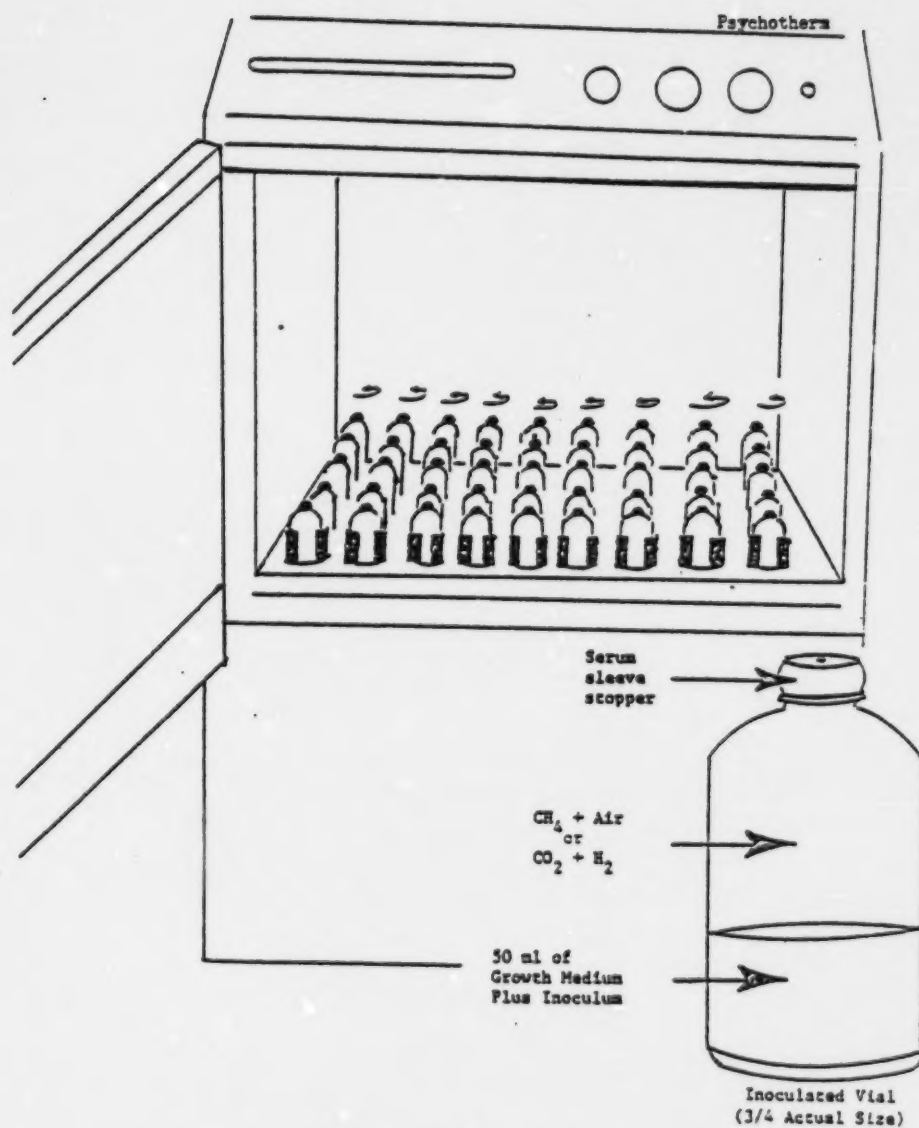


FIGURE D-2 A SKETCH OF AN INOCULATED VIAL AND OF THE SHAKING MACHINE USED IN THE METHANE STUDIES.

TABLE D-1
Components of Culture Vials Used in the Studies

Bacterial Test For	Type of Assay	
	Preliminary Studies To Test the Procedures	Procedures Chosen
Methane-Utilizing Bacteria	Inoculum - Sewage Sludge Soil (From Wooded Area)	Inoculum - Soil Covering the Landfill Topsoil in Control Area
	Media - Brown et al. (1964) Leadbetter and Foster (1953)	Media - Leadbetter and Foster (1953)
	Gases - Natural Gas ^a and Air	Gases - Methane and Air
Methane-Producing Bacteria	Inoculum - Sewage Sludge Soil (From Wooded Area)	Inoculum - Landfill Solid Waste From 5, 7, and 8 $\frac{1}{2}$ Feet Below Soil Surface Soil From Control Area Taken at the Same Depths
	Medium - Methanogenesis Medium	Medium - Methanogenesis Medium
	Gases - N ₂ , CO ₂ , H ₂	Gases - N ₂ , CO ₂ , H ₂

^a - The purified methane had not arrived from the manufacturer at the time of the assay.

period as described above. Nitrogen gas was added to the medium before and after the sterilization. The vials were sterilized with the serum sleeves placed on the top of the opening to allow the air pressure to change during the sterilization. Afterwards the serum sleeves were secured on the mouth of the vials. After cooling and before inoculation of the sample, 0.25 ml of a 50% NaHCO_3 solution was added to each vial together with 0.5 ml of an average overnight culture of Escherichia coli, ATCC 13706. These vials were incubated overnight at 35 C to allow the coliforms to utilize the oxygen (see Figure D-2).

The Inoculation Procedure:

Sewage sludge inoculations were measured by volume, i.e. 5 ml of the undiluted samples and 5 ml of the samples diluted 10^{-1} , 10^{-2} , 10^{-3} , using phosphate buffer (Standard Methods, 1976) were added to each of the vials. Five grams of the soil samples and 5 ml of 10^{-1} , 10^{-2} , 10^{-3} , of the diluted samples were added as indicated in the result tables. The solid waste was weighed in 5 gram amounts and prepared in dilutions with phosphate buffer as described above after homogenization with a Waring blender (at high speed for 15 seconds).

The Incubation Conditions:

After the vials were inoculated, N_2 , CO_2 , or CH_4 purified gases or air, depending upon the experiment, were used to fill the individual vials by adding the gases prior to insertion of the serum sleeve. Generally, however, the gas within the vial was withdrawn with a luer lok syringe and an equal volume of a gas or gases (natural gas, CH_4 , H_2 , or CO_2 , either one or a combination) were added to obtain the desired gaseous atmosphere necessary to given the particular type of methane bacteria, if these bacteria were to be cultured. It was necessary to obtain the correct gaseous atmosphere (Alexander, 1961; Whittenburg et al., 1970; Wolf, 1970; and Foster and Davis, 1966). Namely, methane-using bacteria required CH_4 and air, methane-producing bacteria required anaerobic conditions, CO_2 , and H_2 . For these purposes purified gases were used in the experiments except during the preliminary experiments when the methane gas was unavailable. At that time the natural gas from the laboratory burner jets was used instead. At all other times the Scott Speciality Gases (Bellefonte, Pennsylvania) were used in the culture vials: hydrogen, No. 3-00-100, 99.95% pure; Carbon dioxide, No. 2-3402, 99.8% pure; and methane, No. 2-2562 99.0% pure.

After the gas additions the culture vials were incubated at 35 C for several weeks. These vials were incubated on an incubator-shaking machine. This machine rotated in a one inch diameter circle 148 times per minute (Psychotherm Incubator Shaker, New Brunswick Scientific Co., Inc., New Brunswick, New Jersey). At periodic intervals the vials were removed from the incubator for the purpose of releasing the excess air pressure, to measure the gas-uptake, and to "feed" the required gases to the cultures. Before gases were added to the vials or the gases removed, they were allowed to come to room temperature. Then the gas in the vials containing high gaspressure would be released, and the amount was recorded. Following this, the gas-uptake and gas additions were made. The gas-uptake for a particular vial was always a portion of the gas volume added. Gas-uptake was measured at several times during the incubation period to determine the utilization of gases in the vial. The purified gases listed above were usually "fed" to the vial cultures at least once every two weeks, at which time the gas-uptake was measured. This process involved the filling of a sterile 10 ml syringe with CH_4 , H_2 H_2 (50:50) or H_2 and inserting the needle into the vial. When the gas volume in the vial was decreased, gas from the syringe would be transferred to the vial. This uptake was measured and recorded.

The vial and the attached syringe were laid flat on the table to eliminate the pull of gravity on the syringe plunger. The uptake was recorded and the rest of the gas in the syringe was added to the vial. For example, if 7 ml of gas was taken into the vial by passive action this was gas uptake, and if 10 ml was to be inserted that day, then the other 3 ml of gas would be added to the vial after the amount of uptake was noted.

Gas-Injection Chromotography:

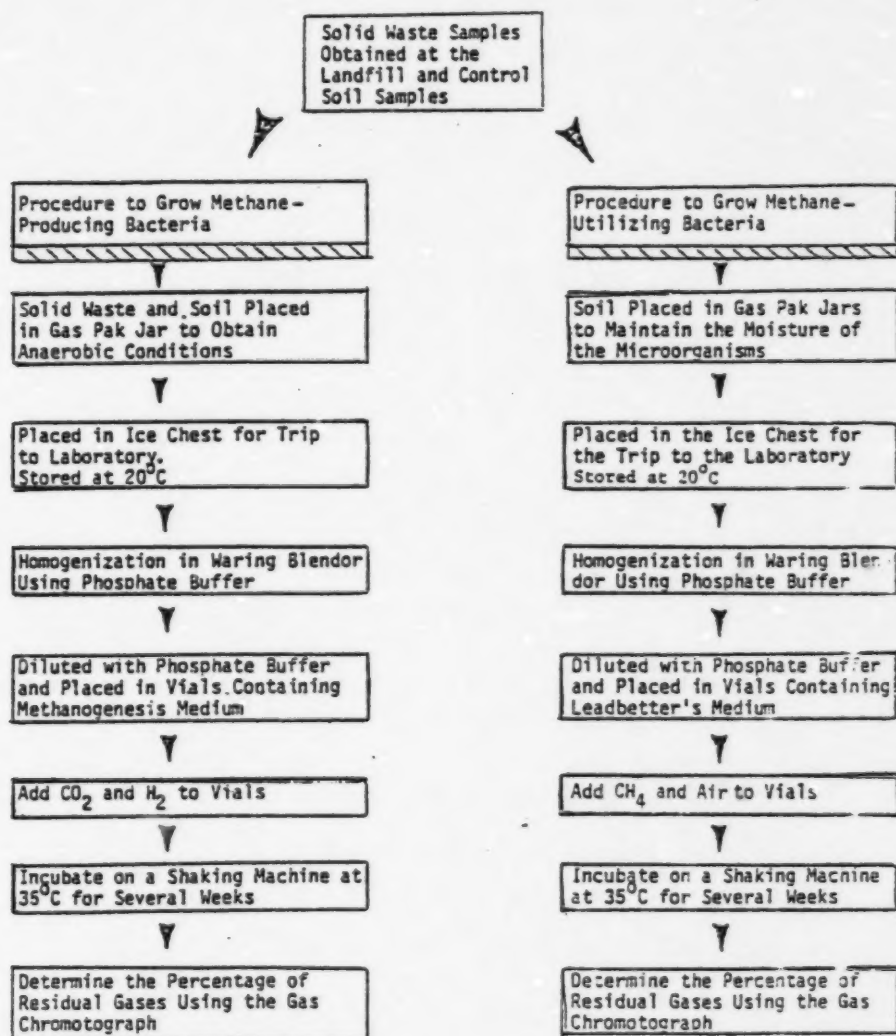
Gas analyses were conducted on a Perkin-Elmer 900 gas chromatograph equipped with a thermal conductivity detector, using helium carrier gas at a flow of 55 cc/minute, with a detector temperature of 250 C and 175 ma current. The column was an Alltech CRT #8700 column (Alltech Associates, Arlington Heights, Illinois) held isothermol at 35 C. Three milliliters volumes of samples were compared to the same volume of a standard gas. The standard used was Standard Scott Gas #9800 (Scott Environmental Technology, Inc., Bellefonte, Pennsylvania) containing CH_4 , CO_2 , CO, O_2 , and N_2 . The analysis was normalized for 100% based on the occurrence of CH_4 , CO_2 , O_2 , and N_2 in the sample (McNair and Bonelli, 1969). A computer program was used for individual sample calculations.

Procedures Used for the Landfill Assays:

After a few trial procedures and after the preliminary assays were completed, final decisions were made on the assays to be used for determining the methane bacteria in the landfill material. These procedures are listed in Figure D-3.

Figure D-3

Flow Chart of the Methane Bacteria Experiments



RESULTS AND DISCUSSION

Preliminary Experiments:

Procedures were designed for measuring methane-utilization and methane-production of environmental samples. The best one was not readily apparent from an examination of the literature. One complicating factor was that great care was needed in performing the experiments because these gases (H_2 and CH_4) used for microbial nutritive purposes were dangerous should large quantities escape into the laboratory atmosphere. Therefore, a closed system was devised. The procedures chosen for the assays needed to be evaluated to determine if they could be used to measure methane-utilization and production. For the preliminary assays sewage sludge and soil from a wooded area were chosen as positive samples because the former contained methane-producing bacteria, and the latter presumably contained both methanogenic bacteria and methane-utilizing bacteria. The description of these preliminary studies and the experimental assays are listed for comparison in Table D-1.

The results of the preliminary studies are shown in Table D-2. In Table D-2 the addition of sewage sludge to Brown's medium in the presence of methane-containing natural gas demonstrated that no methane was used by the bacteria in vials 1, 2, or 3. Because these vials gave negative results,

TABLE D-2

Test of Procedure, Methane Utilization

Culture Vial ^a Number	Inoculum: Sewage Sludge	Indicators of Gas Pressure in Vials Positive, ^b Negative, ^c Pressure	Total Volume of Gases Injected Natural Gas	Final Gas Composition of Culture Vials (Percent)					
				CO ₂	O ₂	N ₂	CH ₄		
<u>Brown's Medium^e</u>									
1	5.0 ml	56 ml	0 ml	90 ml	90 ml	7.3	0.23	33.6	58.9
2	0.5	17	10	90	90	3.5	0.33	38.5	57.7
3	5 x 10 ^{-7.2}	39	0	90	90	3.2	0.21	39.0	57.6
<u>Leadbetter's Medium^g</u>									
4	5.0 grams	0	8	38	88	10.7	1.2	85.2	2.8
5	0.5	0	6	38	88	5.1	1.0	65.1	28.8
6	0.05	0	2	36	86	5.4	0.97	66.9	26.7
7	0.0	0	4	37	87	4.9	0.97	67.4	26.6
8	0.5	0	1	95	25	2.7	2.5	37.9	56.9
9	0.05	0	4	97	27	2.0	0.6	34.7	62.7
10	0.0	0	3	97	27	2.0	0.5	32.9	64.6

a = Incubation at 35 C for 23 days.

b = Gas volumes removed to relieve pressure.

c = Gas-uptake, part of the total gas volume injected.

d = Source of methane (on 12/16/80 the gas contained 12 CO₂, 11.1% O₂, 32.12 N₂, and 57% CH₄).

e = Brown et al. (1964)

f = Not determined

g = Leadbetter and Foster (1953)

no further gas chromatograph analyses were run. Perhaps these bacteria were not present in sewage sludge.

Table D-2 lists the results of two assays involving vials 4 through 10 to which soil was added to the Leadletter's medium; these demonstrated methane-utilization. In both cases, the methane concentration of the first vial (containing the highest concentration of soil) had lower concentration of the gas than the controls, indicating methane use. The only difference between the two assays was that in vials 4 through 7, a low concentration of methane to air (38/88) was used while in the vials 8 through 10 a higher concentration of methane to air (95/25) was used. In conclusion, the Leadletter medium was chosen as an adequate medium as it gave positive results and could, therefore, indicate the presence of methane-users in the landfill samples.

The results of the preliminary tests using the methanogenesis medium inoculated with sewage sludge and soil (from a wooded area) are shown in Table D-3. Note in Table D-3 that gas volumes were removed to relieve the pressure inside the vials, numbered 11 through 16. This pressure was probably due to the production of fermentation products from the nutrients by Escherichia coli. The gas utilization (gas-uptake) of H_2 and CO_2 was most times re-

TABLE D-3

Test of Procedures, Methane Production (Part I)

Culture Vial Number	Inoculum: Sewage Sludge	Indicators of Gas Pressure in Vial: Positive ^b , Negative ^c Pressure	Total Volume of Gases Injected		Final Gas Composition of Culture Vials (Percent)				
			H ₂	CO ₂	CO ₂	O ₂	N ₂	CH ₄	CO
11	5.0 ml	18 ml	0 ml	90 ml	90 ml	34.3	15.0	1.64	23.6
12	0.5	10	2	90	90	62.4	0.12	13.8	3.27
13	5 x 10 ⁻²	10	23	90	90	61.0	0.1	20.8	3.4
14	5 x 10 ⁻³	10	34	90	90	57.1	0.0	22.1	5.21
15 ^e	5.0	22	29	100	100	45.2	0.08	33.2	0.0
16	0.0	5	24	90	90	78.4	0.0	0.0	0.0

a = Incubation at 35 C for 33 days, using Methanogenesis Medium.

b = Gas volumes removed to relieve pressure.

c = Gas-uptake, part of the total gas volume injected.

d = Initially vials were flushed with CO₂. 50 ml of gas was removed from the closed vial, and 50 ml of H₂ was added.e = Escherichia coli was omitted from the vial as a control on the procedure.

TABLE D-3
Test of Procedures, Methane Production (Part II)

Culture Vial Number	Inoculum: Soil from Hooded Area	Indicators of Gas Pressure in Vial: Positive, Negative Pressure ^c	Total Volume of Gases Injected		Final Gas Composition of Culture Vials (Percent)			
			H ₂	CO ₂	CO ₂	O ₂	H ₂	CH ₄
17	5.0	0	14 ml	37 ml	77 ml	76.1	19.4	4.47
18	5 × 10 ⁻¹	0	10	35	75	75.8	24.2	0.0
19	0.0	0	6	33	73	75.9	24.1	0.0
20	5.0	0	20	44	84	77.3	22.4	0.18
21	5 × 10 ⁻¹	0	25	42	82	88.4	11.6	0.0

a = Incubation at 35 °C for 33 days, using Methanogenesis Medium.

b = Gas volumes removed to relieve pressure.

c = Gas-uptake, part of the total gas volume injected.

d = Initially vials were flushed with CO₂. 50 ml of gas was removed from the closed vial, and 50 ml of H₂ was added.

e = *Escherichia coli* was omitted from the vial as a control on the procedure.

lated to the amount of methane produced. Table D-3 results indicated that low levels of methane-production by soil microorganisms can also be indicated by this procedure. In conclusion, the results in these Tables demonstrated that the methanogenesis medium will support the growth of methane-producing bacteria, and, thus, it can be used in the analysis of the landfill solid waste to test for the presence of these bacteria.

The Analysis of the Landfill Soil Cover and the Control Soil for the Presence of Methane-Utilizing Bacteria:

The results are listed in Table D-4. There was no gas pressure produced by the bacteria in the vials. Further, the gas-uptake appeared to be irregular and did not coincide sharply with the percentage of methane-utilized. The gas-uptake of the controls demonstrated no gas-uptake at all. Calculations of the methane-utilized in each culture vial is presented in Table D-5. Each vial had a 100 ml gas volume. The gas concentrations in Table D-4 were expressed as percentages, and these percentages were based on 100. Thus, the gas percentages in Table D-4 can be expressed in gas volumes (milliliters). Therefore, it was possible in Table D-5 to subtract the final methane volumes from the total methane volumes added during the incubation period without regard to Boyle's Law or Charles Law to give approximate

TABLE D-4
Methane-Utilization by Bacteria in the Landfill Soil Cover and the Control Soil.

Culture Vial ^a Number	Soil Concentra- tions Used As Inoculum	Indicators of Gas Pressure in Vials		Total Volume of Gases Injected		Final Gas Composition of Culture Vials (Percent)			
		Positive ^b Pressure	Negative ^c Pressure	CH ₄	Air	CO ₂	O ₂	N ₂	CH ₄
Landfill Soil Taken 1½ Feet Below the Surface									
101	5.0 g	0 ml	9 ml	49 ml	60 ml	6.9	0.67	60.3	32.1
102	0.5	0	14	54	60	4.2	0.66	52.2	42.9
103	5 × 10 ⁻²	0	13	53	60	4.1	0.60	49.2	46.1
104	5 × 10 ⁻³	0	24	64	60	3.7	0.29	48.4	47.7
105	0.0	0	0	40	60	0.0	9.2	47.7	43.1
Control Soil Taken 1½ Feet Below the Surface									
106	5.0 g	0	2	42	60	5.2	0.56	68.5	25.7
107	0.5	0	17	57	60	3.4	0.49	48.7	47.4
108	5 × 10 ⁻²	0	6	46	60	5.0	0.53	55.4	39.1
109	5 × 10 ⁻³	0	13	53	60	3.9	0.75	49.4	46.0
110	0.0	0	0	40	60	0.0	9.1	48.6	42.3

^a = Incubation at 35 C for 37 days, using Leadbetter's Medium (Leadbetter and Foster, 1953).

^b = Gas volumes removed to relieve pressure.

^c = Gas-uptake, part of the total gas volume injected.

Table D-5
Calculations^a of Methane Used in the Culture Vials

	Soil Weights per Vial	Total Volume of Methane Added to Vials	Minus Final Methane Volume ^b	Methane Used
Soil Cover on Landfill	5.0 g	49 ml	32.1 ml	17 ml
	0.5	54	42.9	11
	0.05	53	46.1	7
	0.005	64	47.7	17
	0.0	40	43.1	0
Control Soil	5.0	42	25.7	16
	0.5	57	47.4	15
	0.05	46	39.1	7
	0.005	53	46.0	7
	0.0	40	42.3	0

a - This data came from Table D-4

b - The percentages in Table D-5 are equated with gas volumes in milliliters.

methane volumes used. From Table D-5 it can be seen that the methane utilized by the bacteria in both soil samples were similar, indicating that both of these soil samples utilized methane to about the same degree. In summary, methane-utilization by the bacteria in the soil cover of the landfill was almost identical to that of the control soil. Therefore, one must conclude that the methane which might arise from the biological degradations in the landfill had no apparent effect of increasing the number of methane-users in the soil cover over the landfill.

The Presence of Methane-Bacteria in Landfill Solid Waste and the Soil Controls:

The assays for the presence of methane bacteria in various solid waste levels, and in the control soil are shown in Tables D-6, D-7 and D-8. A generalized view of all of the assay results indicated that the methanogenesis bacteria were present in greater numbers in the solid waste than were present in the control soil, especially at the 5 foot and the 7 foot levels. At the 8-1/2 foot level, the methanogenic bacteria were present in both the control soil and in the landfill solid waste. Apparently the oxygen in the atmosphere was penetrated in the upper layers of the soil controls and was, therefore, inhibitory to the anaerobic methanogenic bacteria. In summary, this work indicated

TABLE D-6
Methane-Production by the Bacteria in the Solid Waste Taken from the Landfill at the 5 Foot Level^a

Culture Vial Number	Weight of Solid Used As Inoculum	Indicators of Gas Pressure in Vials	Total Volume of Gases Injected		Gas Composition of Culture Vials (Percent)				
			H ₂	CO ₂	CO ₂	O ₂	H ₂	CH ₄	
Landfill Solid Waste									
211	5.0 g	81 ml	8 ml	49 ml	70 ml	22.4	0	34.8	42.8
212	0.5	58	1	47	70	22.1	0	33.8	44.0
213	0.05	0	4	55	70	21.1	0	77.4	1.5
214	0.005	0	3	49	70	14.4	0	85.6	<0.14
215	0.0	7	0	30	70	23.1	0	76.9	<0.16
Control Soil ^a									
216	5.0 g	49	0	58	70	11.6	0	84.9	3.5
217	0.5	70	0	50	70	21.3	0	77.2	1.5
218	0.05	5	6	54	70	19.0	0	79.6	1.33
219	0.005	58	0	57	70	17.0	0	82.9	<0.22
220	0.0	0	0	30	70	10.1	<0.4	84.9	<0.21

a = Five foot below the soil surface, using Methanogenesis Medium.

b = Incubation at 35 C for 56 days.

c = Gas volumes removed to relieve pressure.

d = Gas-uptake, part of the total gas volume injected.

Table D-7
Methane Production by the Bacteria in the Solid Waste taken from the Landfill at the 7 Foot Level^a

Culture Vial Number	Weight of Solid Waste Used as Inoculum	Indicators of Gas Pressure in Vials Positive ^c Negative ^d	Total Volume of Gases Injected		Gas Composition of Culture Vials (Percent)				
			N ₂	CO ₂	CO ₂	O ₂	N ₂	CH ₄	
Landfill Solid Waste									
221	5.0 g	66 ml	0 ml	30 ml	70 ml	20.7	0	36.7	42.6
222	0.5	67	12	30	70	20.4	0	39.4	40.2
223	0.05	50	9	30	70	15.9	0	61.4	22.7
224	0.005	0	40	30	70	18.2	0	45.6	36.3
225	0.0	0	0	30	70	26.9	0	72.4	0.71
Control Soil ^a									
226	5.0 g	0	37	30	70	16.2	0	83.8	0.19
227	0.5	0	27	30	70	17.2	0	82.8	0.0
228	0.05	0	18	30	70	9.5	0	90.4	0.0
229	0.005	0	14	30	70	15.8	0	84.2	0.0
230	0.0	2	0	30	70	9.3	0	99.7	0.0

a - Seven feet below the soil surface.

b - Incubation at 35 C for 56 days, using Methanogenesis Medium.

c - Gas volumes removed to relieve pressure.

d - Gas-uptake, part of the total gas volume injected.

Table 1
Methane-Production by the Bacteria in the Solid Waste Taken from the Landfill at the 8½ Foot Level^a

Culture Vial ^b Number	Weight of Solid Wastes Used as Inoculum	Indicators of Gas Pressure in Vials		Total Volume of Gases Injected		Gas Composition of Culture Vials (Percent)			
		Positive Pressure ^c	Negative Pressure ^d	H ₂	CO ₂	CO ₂	O ₂	N ₂	CH ₄
<u>Landfill Solid Waste</u>									
231	5.0 g	0 ml	34 ml	30 ml	70 ml	21.3	0	36.7	42.0
232	0.5	0	18	30	70	20.8	0	35.7	43.5
233	0.05	0	31	30	70	20.2	0	37.9	42.0
234	0.005	0	42	30	70	14.6	0	82.7	2.8
235	0.0	0	7	30	70	8.4	0	91.6	0.12
<u>Control Soil^a</u>									
236	5.0 g	60	11	30	70	17.3	0	38.6	44.0
237	0.5	53	0	30	70	18.8	0	37.1	44.2
238	0.05	0	19	30	70	15.2	0	72.7	12.1
239	0.005	0	34	30	70	29.6	0	70.4	0.0
240	0.0	0	0	30	70	39.5	0	60.4	0.0

a - Eight and one-half feet below the soil surface.

b - Incubation at 35 C for 38 days, using Methanogenesis Medium.

c - Gas volumes removed to relieve pressure.

d - Gas-uptake, part of the total gas volume injected.

the landfill solid waste allowed methane-producing bacteria to survive in layers closer to the soil surface than would be possible without the presence of this waste in the soil. One possible explanation is that the presence of many kinds of bacteria in the solid waste, including the coliforms, probably removed the oxygen diffusing downward into the waste. Also, the presence of the solid waste insured a high moisture level (60%) which undoubtedly aided in the survival of the methanogenic bacteria as compared to soil without these wastes.

CONCLUSIONS AND SIGNIFICANCE OF THE EXPERIMENT

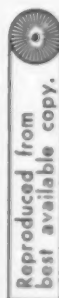
The results of the methane-utilization studies demonstrated that these fecal-indicator bacteria existed in about the same concentration in both the landfill topsoil cover and in the control top soil. This was expected since the topsoil from both sites was probably the same. In the methane-production studies, the bacteria were found in high or significant numbers in all levels of the landfill studied, i.e. the 5, 7, and 8-1/2 foot levels. The control soil contained these bacteria in the lowest level only, and their numbers were slightly less than those in the landfill. Evidently these bacteria are ubiquitous. They probably survive where conditions are favorable and metabolize where substrate is present. Thus, favorable conditions for these bacteria were not present in the upper levels of the soil, but were present only at the 8-1/2 foot level in the control soil, where the oxygen concentration was lower. On the other hand, methane producing bacteria were found in all levels of the landfill. Thus, the discarded waste offered a substrate and optimal condition for methane-production. Also, we must conclude that the oxygen utilizing bacteria created anaerobic conditions, just a foot or so below the soil surface and throughout the entire area of the landfill.

E. MICROBIAL PARAMETERS OF LEACHATE FROM LANDFILLS
AND LYSIMETERS

TABLE E-1. MICROBIAL COUNTS FROM LANDFILL LEACHATE

Quantitative Tests	Landfills					
	Boone County TCIU					
	01/16/77	01/24/78	01/31/78	02/07/78	03/02/78	04/11/78
<u>MPN/100 ml</u>						
Total Coliforms	8	ND ^a	ND	ND	ND	ND
Fecal Coliforms	< 2	ND	ND	ND	ND	ND
Fecal Streptococci	< 2	200	< 200	230	20	50
<u>Agar Plate Counts, CFU/100 ml</u>						
Blood-aerobic	ND	ND	ND	ND	ND	6.7×10^3
Blood-anaerobic	ND	ND	ND	ND	ND	7.0×10^3
Brain heart	ND	ND	ND	ND	ND	1.6×10^3
Eosin Methylene Blue	ND	ND	ND	ND	ND	2.4×10^3
Inhibitory Mold	ND	ND	ND	ND	ND	1.7×10^2
KF Streptococci	ND	ND	ND	ND	ND	ND
Mycoseal *	ND	ND	ND	ND	ND	20
Sabouraud	ND	ND	ND	ND	ND	8.6×10^2
Tellurite	ND	ND	ND	ND	ND	10

* = Not Determined.



[illegible]

TABLE B-3. MICROBIAL COUNTS FROM LANDFILL LEACHATE

Quantitative Tests	Bone County Landfills (Lysimeters)		
	TC-2C		
	03/01/77	01/24/78	02/10/77
<u>PPN/100 ml</u>			
Total Coliforms	< 2	no ^b	< 2
Fecal Coliforms	< 2	no	< 2
Fecal Streptococci	< 2	< 200	no
<u>Agar Plate Counts, CFU/100 ml</u>			
Gluconate	no	no	no
Gluconate	no	no	no
Brain heart	3.1×10^4	no	no
Exon Methylene Blue	< 10	no	no
Inhibitory Mold	no	no	no
CF Streptococci	< 10	no	no
Pyrazol	no	no	no
Embourad	no	no	no
Tellurite	no	no	no

a. n. Not Detected.

TABLE E-5. MICROBIAL COUNTS FROM LANDFILL LEACHATE

Quantitative Tests	Landfills (Lysimeters) Commercial Landfill		
	03/28/78	06/05/78	11/15/77
<u>MPN/100 ml</u>			
Total Coliforms	ND ^a	< 30	< 2
Fecal Coliforms	ND	< 30	< 2
Fecal Streptococci	40	20	2.0×10^2
<u>Agar Plate Counts, CFU/100 ml</u>			
Blood-aerobic	3.0×10^2	1.4×10^3	80
Blood-manaerobic	9.0×10^2	2.6×10^3	ND
Brain heart	2.0×10^2	1.9×10^2	1×10^2
Eosin Methylene Blue	30	1.0×10^2	< 10
Inhibitory Mold	< 10	ND	ND
GP Streptococcal	< 10	< 10	10
Proceal	< 10	ND	ND
Sabouraud	< 10	ND	ND
Tellurite	< 10	< 10	ND

^a = Not Determined

TABLE E-6. MICROBIAL COUNTS FROM LANDFILL LEACHATE

Quantitative Tests	Landfills	
	Commercial Landfill	
	10/22/78	07/24/79
<u>MPN/100 ml</u>		
Total Coliforms	ND ^a	1.8×10^6
Fecal Coliforms	ND	23
Fecal Streptococci	1.7×10^2	3.5×10^6
<u>Agar Plate Counts, CFU/100 ml</u>		
Blood-aerobic	1.9×10^4	6.2×10^6
Blood-anaerobic	3.0×10^3	1.3×10^3
Brain heart	8.0×10^3	2.7×10^6
Basin Methylene Blue	< 10	5.0×10^6
Inhibitory Mold	ND	ND
KP Streptococcal	< 10	ND
Rhizoid	ND	ND
Sabouraud	5.6×10^3	ND
Tellurite	15	ND

a = Not Determined.

Expedited from
test available upon

[illegible]

1004

Downloaded from <http://www.sagepub.com> at
UNIVERSITY OF CALIFORNIA LIBRARY

TABLE 3-9. COCCO POLDS ON FUSAL INDICATORS TESTS^a FROM THE NINETEEN CENTER HILL
LYSIMETER LEACHATES

Leachate ^{b,c} Source	pH	Growth from ethyl violet azide (EVA) broth Standard Methods (1976)		
		Inoculated and Produced Growth:		
		Gross	Colonial Growth	EVA Growth in
		Stain	on K ² Agar	DMZ ^d Broth
Ch-1	9.29	---	---	---
Ch-2	9.13	coast	red	coast
Ch-3	9.07	---	---	coast
Ch-4	9.17	---	---	---
Ch-5	4.70	---	---	---
Ch-6	9.30	---	---	---
Ch-7	9.20	coast	---	coast
Ch-8	9.30	---	---	coast
Ch-9	9.04	coast	---	coast
Ch-10	9.41	---	---	coast
Ch-11	9.18	coast	---	coast
Ch-12	4.09	coast	---	coast
Ch-13	9.46	---	---	coast
Ch-14	9.00	coast	red	coast
Ch-15	9.10	coast	---	---
Ch-16	9.23	---	---	---
Ch-17	9.41	---	---	---
Ch-18	9.16	coast	red	coast
Ch-19	9.03	coast	red	coast

a. = Fusal Indicator? (microbiological) (1976) from, Standard Methods (1976).

b. = Same's data (1976).

c. = See Table 3-1, Section 7 for Fusal Indicator test results.

d. = Microbiological agent.

e. = Growth from indicator broth.

f. = Negative reaction: no growth or no color.

TABLE E-1C. STREPTOCOCCAL LEVELS OF CENTER HILL LYSIMETER LEACHATES (PART 2)

Leachate ^a Source	Fecal Streptococci MPN/100 ml examined by:			
	Standard Methods (1976)		Standard Methods (1976) plus	
			Gram Stain	
	Positive ^b Tubes	Concen- tration	Positive Tubes	Concen- tration
CH-1	(2-0-0-0)	5×10^3	(0-0-0-0)	$< 2 \times 10^3$
CH-2	(1-0-0-0)	2×10^3	(0-0-0-0)	$< 2 \times 10^3$
CH-3	(0-0-0-0)	$< 2 \times 10^3$	(0-0-0-0)	$< 2 \times 10^3$
CH-4	(0-0-0-0)	$< 2 \times 10^3$	(0-0-0-0)	$< 2 \times 10^3$
CH-5	(1-0-0-0)	2×10^3	(0-0-0-0)	$< 2 \times 10^3$
CH-6	(0-0-0-0)	$< 2 \times 10^3$	(0-0-0-0)	$< 2 \times 10^3$
CH-7	(3-0-0-0)	2.3×10^4	(1-0-0-0)	2×10^3
CH-8	(3-3-0-0)	7.9×10^4	(0-0-0-0)	$< 2 \times 10^3$
CH-9	(3-0-0-0)	2.3×10^4	(0-0-0-0)	$< 2 \times 10^3$
CH-11	(3-0-0-0)	2.3×10^4	(1-0-0-0)	2×10^3
CH-12	(3-2-0-0)	7.9×10^4	(3-1-0-0)	3.2×10^4
CH-13	(3-0-0-0)	2.3×10^4	(0-0-0-0)	$< 2 \times 10^3$
CH-14	(2-3-0-0)	1.2×10^4	(1-1-0-0)	4×10^3
CH-15	(0-0-0-0)	2×10^3	(0-0-0-0)	$< 2 \times 10^3$
CH-16	(0-0-0-0)	2×10^3	(0-0-0-0)	$< 2 \times 10^3$
CH-17	(1-0-0-0)	2×10^3	(0-0-0-0)	$< 2 \times 10^3$
CH-18	(0-0-0-0)	2×10^3	(0-0-0-0)	$< 2 \times 10^3$
CH-19	(3-0-0-0)	1.2×10^4	(3-0-0-0)	1.2×10^4

a. R. S. of sample = (a-b-c-d).

b. Values of leachates listed are 10^{10} , 10^{12} , 10^{14} , and 10^{16} , respectively.

c. The positive tubes were tested on the presence of Enterococcus spp., indicating enterococci.

TABLE E-10. STREPTOCOCCAL LEVELS OF CENTER HILL LYSIMETER LEACHATES (PART II)

Leachate ^a Source	Fecal Streptococci MPN/100 ml examined by:			
	Standard Methods (1976)		Standard Methods (1976) plus GRAM STAIN	
	Positive ^b Tubes	Concen- tration	Positive Tubes	Concen- tration
CH-2	(3-0-0)	2.3×10^4	(1-0-0)	4×10^3
CH-3	(3-0-0)	2.3×10^4	(1-0-0)	4×10^3
CH-4	(0-0-0)	$< 1 \times 10^3$	(0-0-0)	$< 1 \times 10^3$
CH-5	(0-0-0)	$< 1 \times 10^3$	(0-0-0)	$< 1 \times 10^3$
CH-6	(0-0-0)	$< 1 \times 10^3$	(0-0-0)	$< 1 \times 10^3$
CH-7	(0-0-0)	$< 1 \times 10^3$	(0-0-0)	$< 1 \times 10^3$
CH-8	(0-0-0)	$< 1 \times 10^3$	(0-0-0)	$< 1 \times 10^3$
CH-9	(0-0-0)	$< 1 \times 10^3$	(0-0-0)	$< 1 \times 10^3$
CH-10	(0-0-0)	$< 1 \times 10^3$	(0-0-0)	$< 1 \times 10^3$
CH-11	(0-0-0)	$< 1 \times 10^3$	(0-0-0)	$< 1 \times 10^3$
CH-12	(0-0-0)	$< 1 \times 10^3$	(0-0-0)	$< 1 \times 10^3$
CH-13	(0-0-0)	$< 1 \times 10^3$	(0-0-0)	$< 1 \times 10^3$
CH-14	(1-0-0)	4×10^3	(1-0-0)	4×10^3
CH-15	(0-0-0)	$< 1 \times 10^3$	(0-0-0)	$< 1 \times 10^3$
CH-16	(0-0-0)	$< 1 \times 10^3$	(1-0-0)	$< 1 \times 10^3$
CH-17	(0-0-0)	$< 1 \times 10^3$	(0-0-0)	$< 1 \times 10^3$
CH-18	(2-0-0)	4×10^3	(2-0-0)	4×10^3
CH-19	(0-0-0)	$< 1 \times 10^3$	(0-0-0)	$< 1 \times 10^3$
CH-20	(0-0-0)	$< 1 \times 10^3$	(0-0-0)	$< 1 \times 10^3$
CH-21	(3-0-0)	4×10^3	(3-0-0)	4×10^3

a. = Date of sample = 10/12/77.

b. = Values of fecal coliform were 10^{10} , 10^{10} , 10^{10} , respectively.

The strep tube test was used.

c. = The positive tubes were based on the presence of Streptococcus spp., indicating fecal coliform.

TABLE B-10. STREPTOCOCCAL LEVELS OF CENTER HILL LYSIMETER LEACHATES (PART 221)

Leachate ^a Source	Fecal Streptococci MPN/100 ml Examined by:			
	Standard Methods(1976)		Standard Methods (1976) plus	
	Positive ^b Tubes	Concen- tration	Gram Stain Positive Tubes	Concen- tration
DH-2	(0-0-0-0)	$< 2 \times 10^2$	(0-0-0-0)	$< 2 \times 10^2$
DH-3	(3-0-0-0)	2.3×10^3	(0-0-0-0)	$< 2 \times 10^2$
DH-4	(0-0-0-0)	$< 2 \times 10^2$	(0-0-0-0)	$< 2 \times 10^2$
DH-5	(0-0-0-0)	$< 2 \times 10^2$	(0-0-0-0)	$< 2 \times 10^2$
DH-6	(0-0-0-0)	$< 2 \times 10^2$	(0-0-0-0)	$< 2 \times 10^2$
DH-7	(0-0-0-0)	$< 2 \times 10^2$	(0-0-0-0)	$< 2 \times 10^2$
DH-8	(1-0-0-0)	4×10^2	(0-0-0-0)	$< 2 \times 10^2$
DH-9	(0-0-1-0)	$< 2 \times 10^2$	(0-0-0-0)	$< 2 \times 10^2$
DH-11	(1-0-0-0)	4×10^2	(0-0-0-0)	$< 2 \times 10^2$
DH-12	(0-0-0-0)	$< 2 \times 10^2$	(0-0-0-0)	$< 2 \times 10^2$
DH-13	(0-1-0-0)	3×10^2	(0-0-0-0)	$< 2 \times 10^2$
DH-14	(1-1-0-0)	7×10^2	(0-0-0-0)	$< 2 \times 10^2$
DH-16	(0-0-0-0)	$< 2 \times 10^2$	(0-0-0-0)	$< 2 \times 10^2$
DH-17	(0-0-0-0)	$< 2 \times 10^2$	(0-0-0-0)	$< 2 \times 10^2$
DH-18	(0-0-0-0)	$< 2 \times 10^2$	(0-0-0-0)	$< 2 \times 10^2$
DH-19	(0-0-1-0)	1.3×10^3	00	00

a. B. Name of Sample. B. 27121/77.

b. B. Values of Positive tubes as 10^{10} , 10^8 , 10^6 , and 10^4 , respectively.

c. B. The positive tubes were tested for presence of Streptococcus fecalis, indicating fecal streptococci.

TABLE E-10. STREPTOCOCCAL LEVELS OF CENTER HILL LYSIMETER LEACHATES (PART IV)

Leachate ^a Source	Fecal Streptococci MPN/100 ml, examined by:			
	Standard Methods (1976)		Standard Methods (1976) plus	
	Positive ^b Tubes	Concen- tration	Gram Stain Positive Tubes	Concen- tration
CH-1	(1-2-1-0)	8	(0-1-1-0)	40
CH-2	(3-5-0-0)	240	(4-5-0-0)	37
CH-3	(3-1-0-0)	33	(0-0-0-0)	< 2
CH-4	(4-4-0-0)	34	(0-0-0-0)	< 2
CH-5	(1-1-1-0)	6	(0-0-0-0)	< 2
CH-6	(0-0-0-0)	< 2	(0-0-0-0)	< 2
CH-7	(3-4-0-0)	17	(0-0-0-0)	< 2
CH-8	(2-3-3-2)	34	(3-1-0-0)	11
CH-10	(0-2-0-0)	30	(0-0-0-0)	< 2
CH-11	(2-4-0-0)	14	(0-0-0-0)	< 2
CH-12	(0-0-0-0)	< 2	(0-0-0-0)	< 2
CH-13	(0-0-0-0)	< 2	(0-0-0-0)	< 2
CH-14	(0-0-0-0)	< 2	(0-0-0-0)	< 2
CH-16	(0-0-0-0)	< 2	(0-0-0-0)	< 2
CH-17	(0-4-0-0)	130	(0-0-0-0)	< 2
CH-18	(0-1-0-0)	20	(0-0-0-0)	< 2

a. = Date of Sample = 11/15/76.

b. = Values of leachate added were 10, 1, 0.1, 10⁻², and 10⁻³, respectively.

c. = The positive tubes were based on no presence of Gram-negative bacilli, indicating streptococci.

TABLE E-11. THE EXAMINATION OF FECAL STREPTOCOCCI MOST-PROBABLE-NUMBER^a(MPN)
ETHYL VIOLET AZIDE TUBES FOR STREPTOCOCCI

Leachate ^b		Growth from Ethyl Violet Azide broth incubated for	
Source	pH	KP ^c Agar	BHI ^d Broth (Gram Stain)
CH-1	5.18	— ^e	—
CH-2	5.28	—	—
CH-3	5.08	—	—
CH-4	5.00	—	—
CH-5	5.09	—	—
CH-6	5.03	—	—
CH-7	5.42	—	exact
CH-8	5.36	—	—
CH-9	5.56	—	—
CH-11	5.21	—	exact
CH-12	5.96	red	exact
CH-13	5.48	—	—
CH-14	5.44	—	exact
CH-15	6.55	—	—
CH-16	5.30	—	—
CH-17	5.42	—	—
CH-18	5.16	—	—
CH-19	5.56	red	exact

a. = Standard Technique (MPN), See Table E-10, Part 5.

b. = Samples taken on 10/21/77.

c. = KP Streptococci agar.

d. = BHI Streptococci broth. No growth or no exact result.

e. = Exact result verification sample.

ETL

TABLE 8-12. FECAL STREPTOCOCCI GROWTH FROM STANDARD METHOD^a (1978) POST-PROBABLY-NUMBER (MPN) ETHYL VIOLET AZIDE TUBES^b

Leachate ^b		Growth from Ethyl Violet Azide broth
Source	pH	Inoculated to DMS ^c Broth
Dr-2	5.06	cccc
Dr-3	5.27	cccc
Dr-4	5.09	---
Dr-5	5.09	cccc
Dr-6	6.95	---
Dr-7	5.21	cccc
Dr-8	5.21	---
Dr-9	5.54	---
Dr-10	5.48	cccc
Dr-11	5.17	---
Dr-12	5.64	---
Dr-13	5.42	---
Dr-14	5.30	cccc
Dr-15	5.16	---
Dr-17	5.00	cccc
Dr-18	5.16	---
Dr-19	5.0 ^d	cccc

a. A. See Table 8-11 for fecal streptococci MPN results.

b. A. Sample from 10/10/78.

c. A. Sample from 10/10/78.

d. A. Negative result.

e. A. See Table 8-11.

TABLE E-13. BACTERIAL CONCENTRATIONS IN LEACHATE FROM THE UNIVERSITY OF CINCINNATI
LYSIMETERS^a (PART I)

Weeks ^b	CFU/ml ^c of Leachate					
	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
4	2.8x10 ⁴	1.5x10 ⁷	5.8x10 ⁵	1.5x10 ⁷	ND ^d	ND
5	2.0x10 ⁶	2.2x10 ⁶	4.7x10 ⁷	8.0x10 ⁷	1.6x10 ⁷	8.5x10 ⁶
6	6.4x10 ⁵	1.5x10 ⁶	1.2x10 ⁷	8.1x10 ⁶	1.4x10 ⁷	1.3x10 ⁷
7	6.9x10 ⁵	3.4x10 ⁶	8.4x10 ⁶	5.7x10 ⁶	7.2x10 ⁶	2.6x10 ⁷
8	2.5x10 ⁶	4.2x10 ⁶	5.5x10 ⁶	ND	4.0x10 ⁶	1.6x10 ⁷
9	1.5x10 ⁶	1.8x10 ⁶	4.0x10 ⁶	3.4x10 ⁵	8.3x10 ⁵	1.0x10 ⁵
10	4.3x10 ⁵	1.0x10 ⁶	2.8x10 ⁵	1.2x10 ⁶	5.6x10 ⁵	1.9x10 ⁶
11	4.3x10 ⁵	2.7x10 ⁶	2.0x10 ⁵	3.2x10 ⁵	4.3x10 ⁵	5.1x10 ⁵
12	7.0x10 ⁵	7.4x10 ⁵	4.0x10 ⁵	7.1x10 ⁵	3.2x10 ⁵	1.4x10 ⁶
13	1.4x10 ⁶	5.4x10 ⁵	7.9x10 ⁵	3.1x10 ⁶	1.5x10 ⁵	6.3x10 ⁶
14	8.6x10 ⁴	3.9x10 ⁵	1.1x10 ⁶	2.8x10 ⁶	2.7x10 ⁴	3.9x10 ⁵
15	3.5x10 ⁵	3.8x10 ⁵	3.2x10 ⁵	3.5x10 ⁶	1.0x10 ⁵	3.2x10 ⁶

a = Lysimeter contents: UC-A = Sewage Sludge

UC-B = Sewage Sludge + Municipal Solid Waste

UC-C = Municipal Solid Waste

UC-D = Municipal Solid Waste

UC-E = Hospital Waste + Municipal Solid Waste

UC-F = Hospital Waste

b = Weeks after lysimeter construction.

c = Determined using plate count agar incubated aerobically at 37°C for 48 hours.

d = Not Determined.



TABLE 2-13. BACTERIAL CONCENTRATIONS IN LEACHATE FROM THE UNIVERSITY OF CINCINNATI
LYSIMETERS^a (PART II)

Weeks ^b	CFU/ml ^c of Leachate					
	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
17	9.7×10^5	2.5×10^5	8.2×10^5	1.6×10^5	5.3×10^4	2.8×10^6
19	2.3×10^4	1.0×10^5	1.3×10^5	1.1×10^6	2.8×10^3	1.5×10^3
22	6.1×10^5	8.8×10^4	6.0×10^6	2.1×10^7	5.1×10^5	2.0×10^5
27	7.3×10^5	5.7×10^4	5.3×10^5	5.2×10^6	5.0×10^4	5.4×10^4
31	7.5×10^5	5.8×10^3	1.0×10^6	9.5×10^5	1.1×10^5	6.3×10^5
35	6.9×10^5	5.1×10^5	3.5×10^4	4.5×10^6	3.2×10^5	1.7×10^6
40	3.9×10^5	3.1×10^4	1.5×10^6	2.2×10^6	1.8×10^5	6.9×10^5
50	1.3×10^6	6.2×10^5	6.5×10^5	2.4×10^6	1.3×10^6	2.3×10^6
54	2.9×10^6	1.8×10^5	7.3×10^5	2.3×10^6	2.3×10^5	3.0×10^4
58	1.9×10^6	9.3×10^3	2.5×10^5	2.3×10^4	1.2×10^4	1.6×10^4
100	1.0×10^8	1.4×10^6	1.2×10^8	2.2×10^7	2.8×10^7	3.1×10^4

a = Lysimeter contents: UC-A = Sewage Sludge

UC-B = Sewage Sludge + Municipal Solid Waste

UC-C = Municipal Solid Waste

UC-D = Municipal Solid Waste

UC-E = Hospital Waste + Municipal Solid Waste

UC-F = Hospital Waste

b = Weeks after lysimeter construction.

c = Determined using plate count agar incubated aerobically at 37°C for 48 hours.

TABLE E-14. MICROBIAL LEVELS IN LEACHATE FROM THE UNIVERSITY OF CINCINNATI LYSIMETERS^a
AS DETERMINED BY AEROBIC AND ANAEROBIC BLOOD AGAR PLATES (PART I)

Weeks ^b	Aerobic (Anaerobic) CFU/ml ^c of Leachate					
	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
4	1.6x10 ⁴ (1.0x10 ³)	4.5x10 ⁵ (2.6x10 ⁸)	2.2x10 ⁵ (2.3x10 ⁷)	1.5x10 ⁷ (6.9x10 ⁷)	ND ^d (ND)	ND (ND)
5	1.5x10 ⁵ (1.5x10 ⁵)	2.4x10 ⁶ (1.3x10 ⁷)	1.8x10 ⁷ (4.4x10 ⁷)	1.5x10 ⁷ (6.7x10 ⁷)	1.0x10 ⁷ (3.1x10 ⁷)	2.2x10 ⁷ (4.5x10 ⁷)
8	(8.1x10 ⁴)	(2.1x10 ⁶)	(3.0x10 ⁷)	(2.8x10 ⁷)	(1.3x10 ⁷)	(2.0x10 ⁷)
9	7.5x10 ⁴ (6.6x10 ³)	1.0x10 ⁶ (3.1x10 ⁴)	7.9x10 ⁵ (1.6x10 ⁶)	1.2x10 ⁵ (3.4x10 ⁵)	1.4x10 ⁵ (8.8x10 ⁵)	1.0x10 ⁵ (1.3x10 ⁶)
10	(2.2x10 ⁴)	(1.1x10 ⁵)	(1.4x10 ⁵)	(2.1x10 ⁵)	(1.2x10 ⁶)	(1.1x10 ⁵)
12	(5.0x10 ³)	(1.3x10 ⁵)	(3.2x10 ⁵)	(5.0x10 ⁴)	(7.4x10 ⁴)	(1.5x10 ⁵)
13	1.9x10 ⁵ (6.3x10 ³)	1.2x10 ⁶ (1.1x10 ⁵)	6.8x10 ⁵ (8.0x10 ⁵)	2.7x10 ⁶ (5.6x10 ⁵)	7.6x10 ⁴ (3.1x10 ⁵)	1.2x10 ⁵ (2.1x10 ⁶)
14	(3.0x10 ³)	(1.7x10 ⁵)	(4.6x10 ⁵)	(2.4x10 ⁵)	(1.5x10 ⁵)	1.0x10 ⁶
15	(4.0x10 ⁴)	(6.0x10 ⁴)	(1.2x10 ⁵)	(1.4x10 ⁵)	(6.0x10 ⁴)	4.2x10 ⁵
17	3.7 x 10 ⁵ (3.8x10 ⁵)	6.3x10 ⁴ (ND)	7.9x10 ⁵ (9.4x10 ⁴)	1.8x10 ⁷ (3.1x10 ⁵)	3.0x10 ⁴ (3.0x10 ⁵)	6.2x10 ⁴ (1.6x10 ⁵)

a = Lysimeter contents: UC-A = Sewage Sludge

UC-B = Sewage Sludge + Municipal Solid Waste

UC-C = Municipal Solid Waste

UC-D = Municipal Solid Waste

UC-E = Hospital Waste + Municipal Solid Waste

UC-F = Hospital Waste

b = Weeks after lysimeter construction.

c = Determined using Blood Agar Plates incubated aerobically and anaerobically using a GasPak Jar at 37°C for 48 hours.

d = Not Determined.



TABLE E-14. MICROBIAL LEVELS IN LEACHATE FROM THE UNIVERSITY OF CINCINNATI LYSIMETERS^a
AS DETERMINED BY AEROBIC AND ANAEROBIC BLOOD AGAR PLATES (PART II)

Weeks ^b	Aerobic (Anaerobic) CFU/ml ^c of Leachate					
	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
27	1.5x10 ⁵	4.0x10 ⁴	8.8x10 ⁴	7.6x10 ⁶	1.7x10 ⁴	1.4x10 ⁴
	(6.2x10 ⁴)	(1.0x10 ⁴)	(3.0x10 ³)	(4.0x10 ⁴)	(3.1x10 ⁴)	(7.5x10 ⁴)
31	3.3x10 ⁵	5.4x10 ⁴	6.0x10 ⁶	5.0x10 ⁵	1.1x10 ⁴	1.9x10 ⁵
	(1.4x10 ⁵)	(2.7x10 ³)	(8.2x10 ⁵)	(1.7x10 ⁵)	(7.0x10 ⁵)	(7.0x10 ⁴)
35	1.9x10 ⁵	2.7x10 ⁴	1.7x10 ⁵	4.1x10 ⁶	6.1x10 ⁴	1.3x10 ⁶
	(3.6x10 ⁵)	(1.2x10 ⁵)	(1.9x10 ⁵)	(3.4x10 ⁵)	(1.5x10 ⁶)	(2.3x10 ⁵)
40	2.4x10 ⁵	4.2x10 ⁴	1.2x10 ⁶	7.7x10 ⁵	1.7x10 ⁴	6.3x10 ⁴
	(1.7x10 ⁵)	(3.9x10 ⁴)	(3.8x10 ⁴)	(8.8x10 ⁴)	(2.4x10 ⁵)	(5.4x10 ⁵)
54	3.6x10 ⁶	1.1x10 ⁵	2.9x10 ⁵	2.3x10 ⁴	1.5x10 ⁴	1.4x10 ⁴
	(1.0x10 ⁶)	(7.0x10 ¹)	(3.6x10 ³)	(5.6x10 ⁴)	(2.3x10 ⁴)	(7.6x10 ⁴)
100	1.2x10 ⁸	1.3x10 ⁶	1.6x10 ⁶	1.4x10 ⁷	9.5x10 ⁵	3.3x10 ⁵
	(1.2x10 ⁸)	(3.3x10 ⁶)	(3.2x10 ⁶)	(4.0x10 ⁶)	(1.7x10 ⁶)	(2.7x10 ⁶)

a = Lysimeter contents: UC-A = Sewage Sludge

UC-B = Sewage Sludge + Municipal Solid Waste

UC-C = Municipal Solid Waste

UC-D = Municipal Solid Waste

UC-E = Hospital Waste + Municipal Solid Waste

UC-F = Hospital Waste

b = Weeks after lysimeter construction.

c = Determined using Blood Agar Plates incubated aerobically and anaerobically using a GasPak Jar at 37°C for 48 hours.

TABLE E-15. THE CONCENTRATION OF NON-SPORE-FORMING, GRAM POSITIVE, ROD-SHAPED BACTERIA^a GROWING ON TELLURITE AGAR FROM THE UNIVERSITY OF CINCINNATI LYSIMETER^b LEACHATE

CFU ^d /ml of Leachate from Lysimeters:						
Weeks ^c	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
4	7.5x10 ³	3.4x10 ⁴	7.4x10 ³	5.7x10 ⁴	ND ^e	ND
5	2.2x10 ⁴	2.5x10 ⁴	9.7x10 ⁴	9.2x10 ⁴	2.6x10 ⁴	1.1x10 ⁵
9	2.8x10 ⁴	1.9x10 ⁴	4.7x10 ⁴	1.3x10 ⁴	5.1x10 ⁴	1.7x10 ⁴
13	1.1x10 ⁴	1.6x10 ⁴	7.8x10 ⁴	4.5x10 ⁵	1.7x10 ⁴	5.9x10 ⁴
17	1.3x10 ⁴	5.8x10 ³	2.9x10 ⁴	3.0x10 ⁴	1.3x10 ⁴	8.1x10 ⁴
22	4.2x10 ⁵	1.0x10 ⁴	1.4x10 ⁵	1.1x10 ⁶	5.7x10 ⁵	1.4x10 ⁴
31	1.7x10 ³	3.0x10 ²	5.3x10 ³	2.0x10 ²	6.2x10 ⁴	1.9x10 ⁴
35	5.8x10 ²	1.3x10 ⁵	1.1x10 ⁴	1.9x10 ³	1.2x10 ³	1.6x10 ³
40	3.0x10 ²	8.0x10 ²	1.0x10 ⁴	1.0x10 ²	4.0x10 ²	8.0x10 ³
54	< 10	2.5x10 ²	4.0x10 ²	< 10	1.3x10 ¹	2.7x10 ³
100	4.0x10 ¹	2.9x10 ⁴	4.0x10 ³	1.6x10 ⁴	6.4x10 ⁴	< 10 ³

a = Examples are Corynebacterium sp., Kurtzia sp., and Listeria sp.

b = Lysimeter contents: UC-A = Sewage Sludge; UC-B = Sewage Sludge + Municipal Solid Waste; UC-C = Municipal Solid Waste; UC-D = Municipal Solid Waste; UC-E = Hospital Waste + Municipal Solid Waste; and UC-F = Hospital Waste.

c = Weeks after construction.

d = Determined by growth on tellurite agar plates grown at 37°C for 48 hours.

e = Not Determined.

TABLE E-16. THE CONCENTRATIONS OF FUNGI^a FROM THE UNIVERSITY OF CINCINNATI LYSIMETER^b
LEACHATE

Weeks ^c	CFU ^d /100 ml of Leachate from Lysimeters					
	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
4	1.6x10 ⁶	1.7 x 10 ⁸	2.9x10 ⁷	6.1x10 ⁷	ND	ND
5	9.8x10 ⁷	1.2x10 ⁸	3.2x10 ⁸	3.5x10 ⁸	1.7x10 ⁷	1.9x10 ⁸
7	9.8x10 ⁷	2.9x10 ⁸	4.5x10 ⁷	3.5x10 ⁸	5.1x10 ⁷	6.5x10 ⁸
9	2.1x10 ⁷	1.1x10 ⁸	1.4x10 ⁷	9.0x10 ⁷	3.5x10 ⁷	3.0x10 ⁸
11	3.5x10 ⁷	5.5x10 ⁷	1.5x10 ⁷	2.6x10 ⁷	6.6x10 ⁷	5.6 x 10 ⁶
13	2.2x10 ⁷	4.9x10 ⁷	2.2x10 ⁷	6.6x10 ⁷	4.6x10 ⁷	5.2x10 ⁶
17	3.8x10 ⁷	1.4x10 ⁷	7.3x10 ⁷	9.3x10 ⁸	2.2x10 ⁷	7.0x10 ⁸
22	1.4x10 ⁶	3.2x10 ⁶	1.5x10 ⁶	1.1x10 ⁷	1.1x10 ⁷	1.0x10 ⁷
31	2.8x10 ⁵	6.0x10 ⁴	5.7x10 ⁵	2.8x10 ⁶	7.5x10 ⁵	2.3x10 ⁶
35	1.5x10 ⁵	5.3x10 ⁵	9.3x10 ⁵	1.2x10 ⁶	6.3x10 ⁶	6.3x10 ⁶
40	4.7x10 ⁶	9.0x10 ⁴	2.3x10 ⁶	4.4x10 ⁴	1.5x10 ⁶	9.4x10 ⁶
54	1.0x10 ⁷	1.1x10 ⁶	5.0x10 ⁵	1.6x10 ⁶	3.3x10 ⁵	2.7x10 ⁶
100	3.1x10 ⁶	4.2x10 ⁷	4.2x10 ⁷	1.7x10 ⁶	3.5x10 ⁵	1.9x10 ⁶

a = Includes yeasts and molds.

b = Lysimeter contents: UC-A = Sewage Sludge

UC-B = Sewage Sludge + Municipal Solid Waste

UC-C = Municipal Solid Waste

UC-D = Municipal Solid Waste

UC-E = Hospital Waste + Municipal Solid Waste

UC-F = Hospital Waste

c = Weeks after construction.

d = Determined by plating dilutions of leachate on Sabouraud Dextrose agar and incubating them at room temperature for one week.

TABLE E-17. THE CONCENTRATION OF STREPTOCOCCI GROWING ON KF STREPTOCOCCAL AGAR^a
FROM THE UNIVERSITY OF CINCINNATI LYSIMETER LEACHATES.

Weeks ^c	CFU/100 ml of Leachate from Lysimeters ^b					
	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
4	-- ^d	2.35x10 ⁶	2.0x10 ⁶	1.7x10 ⁶	--	--
5	--	2.3x10 ⁶	1.4x10 ⁷	1.7x10 ⁷	6.5x10 ⁵	9.0x10 ⁶
9	5.0x10 ³	2.5x10 ⁵	4.0x10 ⁶	2.6x10 ⁵	3.8x10 ⁶	5.0x10 ³
13	5.0x10 ²	6.0x10 ⁴	1.0x10 ⁵	1.0x10 ⁵	1.3x10 ⁵	6.6x10 ⁴
17	< 1x10 ³	5.0x10 ²	1.3x10 ⁴	3.0x10 ³	1.9x10 ⁴	5.0x10 ²
31	< 10	30	10	10	10	< 10

a = From KF Streptococcal Agar Plate Counts (CFU/100 ml Leachate).

b = University of Cincinnati, Cincinnati, Ohio.

c = Weeks after lysimeter construction.

d = Not determined.

F. CHEMICAL PARAMETERS OF LEACHATE FROM LANDFILLS
AND LYSIMETERS

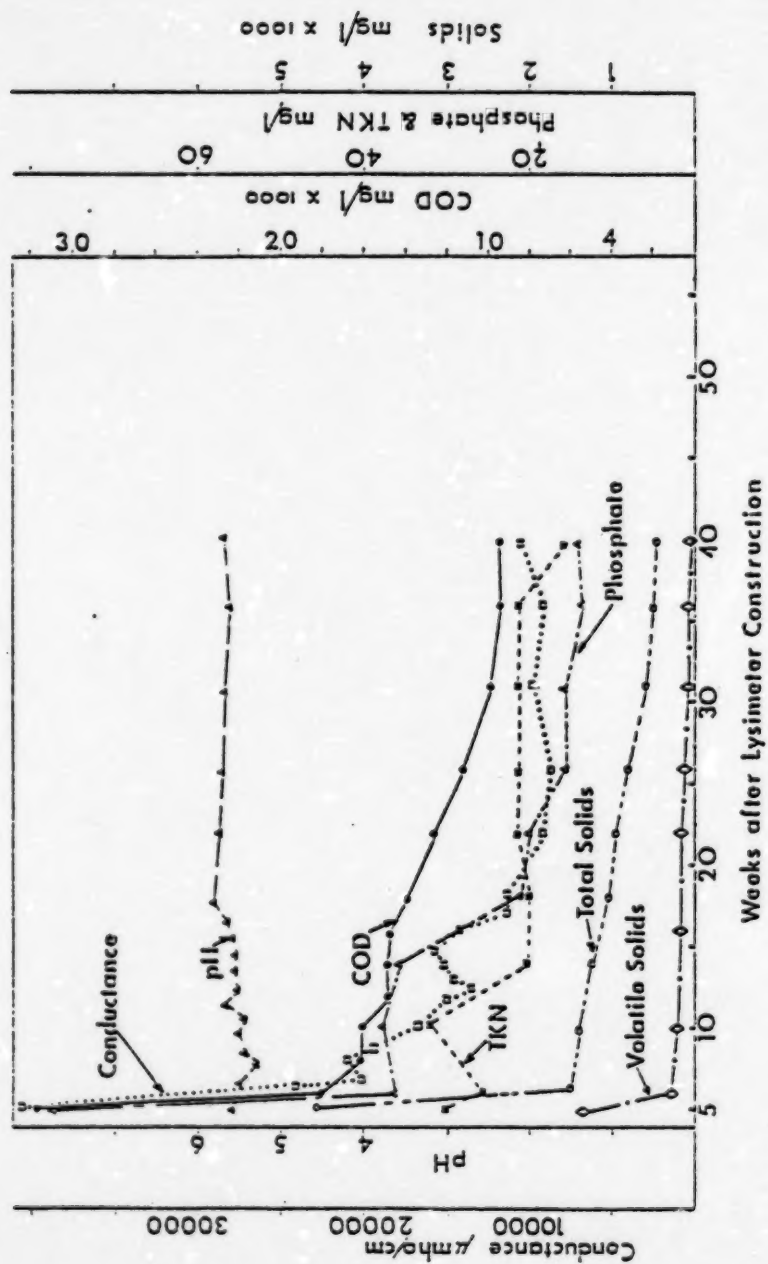


FIGURE F-1. CHEMICAL ANALYSIS OF LEACHATES FROM LYSIMETER UC-13.

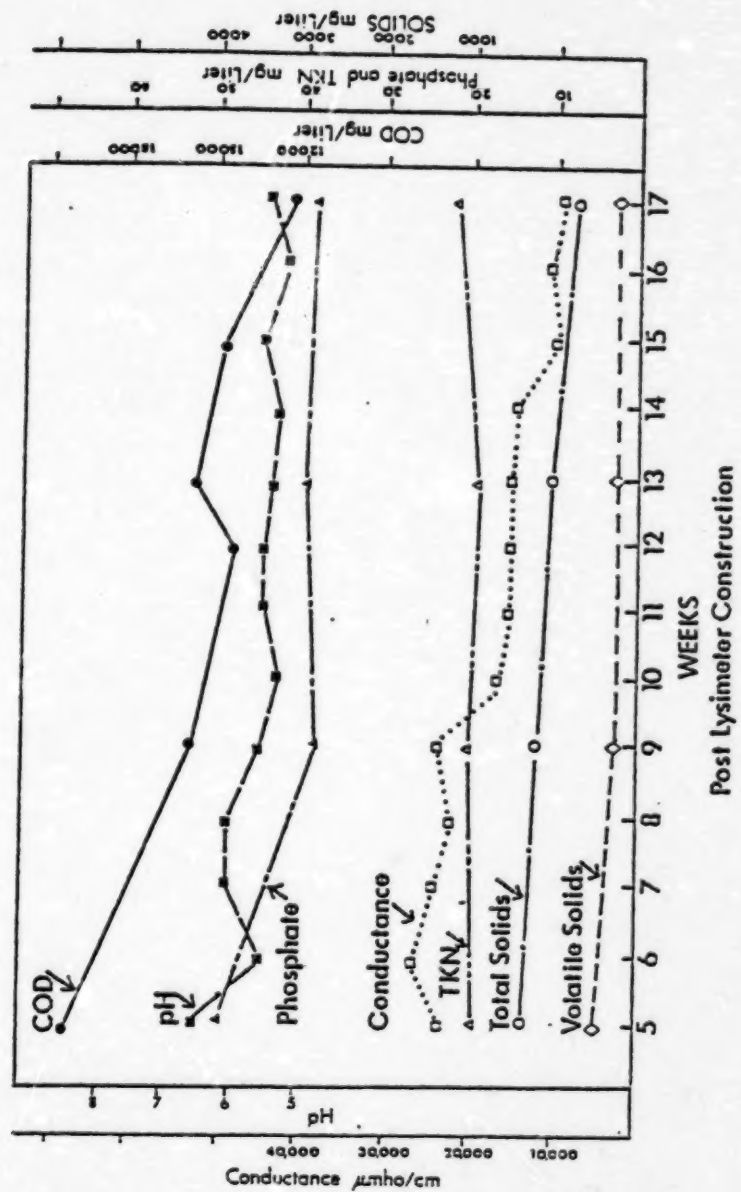


FIGURE F-2. CHEMICAL ANALYSIS OF LEACHATES FROM LABORATORY LYSIMETER UC-2

TABLE F-1. CHEMICAL PARAMETERS OF LEACHATES FROM BOONE COUNTY LANDFILLS

Parameters	Chemical Levels in Leachates from the Following Landfills (Lysimeters)				
	TC1U		TC2C		TC2D
	04/11/78	05/30/78	04/11/78	05/30/78	11/15/78
COD ^{a,b} (mg/l)	1,800	1,580	20,730	21,000	14,323
Conductance ^b (unhos/cm)	ND ^c	ND	ND	ND	9,449
Kjeldahl, total ^b (mg/l)	98	131	392	400	472
pH ^d	6.65	ND	5.17	4.29	5.69
TOC (mg/l) ^e	665	1,550	7,520	5,560	710
Phosphate, total ^d (mg/l)	1.5	2.1	4.5	6.5	7.5
Solids, total ^b (mg/l)	2,773	2,421	9,433	9,737	11,100
Solids, volatile ^b (mg/l)	853	879	4,706	4,993	4,710

a = Chemical oxygen demand.

b = Determined by the EPA Methods for Chemical Analysis of Water and Wastes (1974).

c = Not Determined.

d = Determined by Chian and DeWalle (1975).

e = Standard Methods (1976).Reproduced from
best available copy.

TABLE F-2. CHEMICAL PARAMETERS OF LEACHATES FROM THE CENTER HILL LYSIMETERS CH-9 and CH-19

Parameter	Landfills (Lysimeters)				
	CH-9			CH-19	
	11/15/78	03/20/78	05/31/78	09/19/77	10/17/77
COD ^{a,b}	31,149	24,360	26,080	30,700	27,066
Conductance (umhos/cm)	13,339	14,173	ND ^c	ND	18,298
Kjeldahl, total ^b (mg/l)	900	686	728	ND	998
pH ^d	5.66	5.71	5.34	5.23	5.29
TOC (mg/l) ^e	ND	9,648	6,780	ND	376
Phosphate, total ^d (mg/l)	11.0	7.5	8.8	ND	2.55
Solids, total ^b (mg/l)	19,623	16,913	14,439	ND	21,336
Solids, Volatile ^b (mg/l)	10,827	8,860	7,464	ND	11,833

a = Chemical oxygen demand.

b = Determined by the EPA Methods for Chemical Analysis of Water and Wastes (1974).

c = Not Determined.

d = Determined by Chian and DeWalle (1975).

e = Standard Methods (1976).

TABLE F-3. CHEMICAL PARAMETERS OF LEACHATE FROM A CENTER HILL LYSIMETER CH-19
AND THE COMMERCIAL LANDFILL.

Parameters	CH-19			COMMERCIAL LANDFILL		
	11/15/78	03/20/78	05/31/78	11/17/77	03/27/78	06/05/78
COO ^{a,b} (mg/l)	35,531	34,110	30,090	687	666	580
Conductance ^b (umhos/cm)	13,339	14,173	ND ^c	9,071	9,449	ND
Kjeldahl, total ^b (mg/l)	1,142	1,092	920	422	303	383
pH ^d	5.13	5.23	4.83	7.35	7.23	7.3
TOC (mg/l) ^e	1,660	13,680	12,400	115	575	480
Phosphate, total ^d	25.5	21.0	17.0	4.5	2.8	2.4
Solids, total ^b (mg/l)	21,336	21,040	16,019	3,946	3,366	2,822
Solids, volatile ^b (mg/l)	11,833	10,973	8,775	793	453	230

a = Chemical oxygen demand.

b = Determined by the EPA Methods for Chemical Analysis of Water and Wastes (1974).

c = Not Determined.

d = Determined by Chian and DeWalle (1975).

e = Standard Methods (1976).

TABLE F-4. CONDUCTANCE OF LEACHATES FROM THE UNIVERSITY OF CINCINNATI LYSIMETERS^a

Weeks ^b	Conductance ^c (umhos/cm) of Leachates from Lysimeters					
	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
4	32,760	41,890	22,640	30,030	ND ^d	ND
5	16,696	23,375	23,375	23,375	23,375	7,304
6	21,400	20,020	15,060	22,810	27,260	22,640
7	21,060	21,060	15,010	21,700	25,070	21,780
8	18,000	19,500	13,470	22,100	23,460	20,080
9	16,370	16,960	12,860	22,360	24,280	21,630
10	15,460	14,970	12,900	15,470	15,800	16,900
11	12,090	13,900	13,200	14,290	15,050	16,785
12	11,400	14,250	12,800	12,800	14,800	16,273
13	11,050	14,500	13,260	13,275	13,940	15,970
14	9,890	15,200	13,100	14,080	13,290	15,440
15	10,280	13,800	14,200	12,200	9,650	15,680
16	6,430	11,200	15,600	11,600	11,200	13,250
17	4,480	10,800	12,800	8,600	9,250	13,240

a = The lysimeter contents are: UC-A = Sewage Sludge

UC-B = Sewage Sludge + Municipal Solid Waste

UC-C = Municipal Solid Waste

UC-D = Municipal Solid Waste

UC-E = Hospital Waste + Municipal Solid Waste

UC-F = Hospital Waste

b = Weeks after lysimeter construction.

c = Determined by Standard Methods (1976)

d = Not Determined.

TABLE F-5. COD LEVELS IN LEACHATES FROM THE UNIVERSITY OF CINCINNATI LYSIMETERS^a

Weeks ^b	Mg/l COD ^c in Leachates from Lysimeters					
	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
4	20,682	31,876	21,647	32,164	— ^d	—
5	16,187	18,178	14,099	22,189	27,182	14,865
7	18,762	16,022	13,299	17,611	17,882	12,094
9	12,078	16,287	13,199	18,079	21,286	10,760
11	9,600	15,220	13,460	17,263	19,194	10,650
13	9,350	15,300	14,000	17,240	22,746	14,290
15	10,280	13,800	14,200	12,200	9,650	15,680
17	6,430	14,280	14,500	14,280	16,190	16,840
22	6,527	10,509	9,703	12,095	13,257	11,863
27	5,056	9,330	7,523	12,310	11,628	11,847
31	4,759	8,573	7,246	10,981	10,717	11,553
35	4,716	8,409	7,198	10,079	10,378	10,986
40	4,453	8,708	6,848	9,435	10,392	10,117
50	4,800	9,501	5,299	7,526	11,110	10,580
100	1,160	21,860	10,690	10,260	7,390	2,500

a = The lysimeter contents are: UC-A = Sewage Sludge

UC-B = Sewage Sludge + Municipal Solid Waste

UC-C = Municipal Solid Waste

UC-D = Municipal Solid Waste

UC-E = Hospital Waste + Municipal Solid Waste

UC-F = Hospital Waste

b = Weeks after lysimeter construction.

c = Determined by the EPA Methods for Chemical Analysis of Water and Wastes (1974).

d = Not Determined.

TABLE F-5. KJELDAHL NITROGEN OF LEACHATES FROM THE UNIVERSITY OF CINCINNATI LYSIMETERS^a

Weeks ^b	Total Kjeldahl Nitrogen ^c (mg/l) of Leachates from Lysimeters:					
	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
4	26.8	31.6	42.6	29.7	ND ^d	ND
5	ND	ND	ND	26.1	20.1	19.7
9	19.8	20.2	25.8	22.2	20.8	19.1
13	12.1	19.7	26.4	21.7	19.6	19.8
17	18.6	8.6	25.2	15.2	22.2	13.6
22	14.7	22.0	28.2	19.0	28.2	12.5
27	14.8	23.5	32.8	26.8	22.4	13.7
31	10.4	23.4	29.3	27.2	22.3	11.5
35	9.25	24.0	27.9	22.4	30.6	9.8
40	8.6	18.9	23.5	26.4	30.7	10.3
50	9.3	16.9	13.1	21.3	24.6	6.5
100	800	38	15	25	20	5

a = The lysimeter contents are: UC-A = Sewage Sludge

UC-B = Sewage Sludge + Municipal Solid Waste

UC-C = Municipal Solid Waste

UC-D = Municipal Solid Waste

UC-E = Hospital Waste + Municipal Solid Waste

UC-F = Hospital Waste

b = Weeks after lysimeter construction.

c = Determined by the EPA Methods for Chemical Analysis of Water and Wastes (1974).

d = Not Determined.

TABLE F-7. pH OF LEACHATES FROM THE UNIVERSITY OF CINCINNATI LYSIMETERS^a

Weeks ^b	pH ^c of Leachates from Lysimeters:					
	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
4	5.43	5.55	4.94	5.07	ND ^d	ND
5	5.47	5.27	5.05	6.02	6.55	4.66
6	5.53	5.48	5.83	5.61	5.51	5.42
7	5.51	5.27	5.81	5.61	6.15	5.79
8	5.53	5.36	5.77	5.61	6.15	5.79
9	5.52	5.41	5.86	5.62	5.68	5.39
10	5.51	5.36	5.30	5.36	5.44	5.55
11	5.58	5.42	5.25	5.22	5.61	5.57
12	5.69	5.37	5.32	5.28	5.59	5.57
13	5.72	5.41	5.39	5.26	5.43	5.62
14	5.68	5.44	5.29	5.39	5.41	5.59
15	5.47	5.53	5.28	5.41	5.62	5.48
16	5.29	5.61	5.55	5.17	5.26	5.33
17	5.39	5.72	5.59	5.29	5.51	5.59

a = The lysimeter contents are: UC-A = Sewage Sludge

UC-B = Sewage Sludge + Municipal Solid Waste

UC-C = Municipal Solid Waste

UC-D = Municipal Solid Waste

UC-E = Hospital Waste + Municipal Solid Waste

UC-F = Hospital Waste

b = Weeks after lysimeter construction.

c = Determined by the EPA Methods for Chemical Analysis of Water and Wastes (1974).

d = Not Determined.

Table F-8. PHOSPHORUS LEVELS IN LEACHATES FROM THE UNIVERSITY OF CINCINNATI
EXPERIMENTAL LYSIMETERS^a

Weeks ^b	mg/l Phosphorus ^c in Leachates from Lysimeters:					
	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
4	62.7	80.1	82.8	84.6	— ^d	—
5	26.2	37.3	36.1	42.6	51.8	46.3
9	24.6	40.1	32.7	38.2	39.2	42.1
13	18.1	36.2	30.8	36.8	41.4	48.7
17	10.6	22.8	31.3	21.2	39.7	42.6
22	9.6	19.7	30.6	17.5	35.6	38.8
27	4.15	15.9	32.4	15.0	30.5	38.7
31	1.8	16.9	29.3	15.4	28.4	36.1
35	ND	13.9	26.2	16.1	22.5	30.9
40	ND	14.6	20.8	16.0	19.5	27.4
50	ND	9.1	5.75	15.8	7.9	8.2
100	6.4	21.2	21.0	22.0	12.0	2.3

a = The lysimeter contents are: UC-A = Sewage Sludge

UC-B = Sewage Sludge + Municipal Solid Waste

UC-C = Municipal Solid Waste

UC-D = Municipal Solid Waste

UC-E = Hospital Waste + Municipal Solid Waste

UC-F = Hospital Waste

b = Weeks after lysimeter construction.

c = Determined by method described in Chian and DeWalle (1975).

d = Not Determined.

TABLE F-9. TOTAL SOLIDS OF LEACHATES FROM THE UNIVERSITY OF CINCINNATI LYSIMETERS^a

Weeks ^b	Total Solids ^c (mg/l) of Leachates from Lysimeters:					
	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
3	1151	555	2205	511	ND ^d	ND!!!
4	2242	4686	5504	4811	ND ^d	ND
5	1401	1676	1467	1327	1486	1529
9	880	1496	1450	962	1146	1496
13	560	1230	1530	1170	1005	1620
17	490	1080	1280	1150	990	1440
22	355	940	980	1050	700	1055
27	275	775	820	950	625	945
31	230	575	605	877	556	852
35	196	485	566	622	321	576
40	177	427	398	562	295	523
50	172	356	227	406	312	500

a = The lysimeter contents are: UC-A = Sewage Sludge

UC-B = Sewage Sludge + Municipal Solid Waste

UC-C = Municipal Solid Waste

UC-D = Municipal Solid Waste

UC-E = Hospital Waste + Municipal Solid Waste

UC-F = Hospital Waste

b = Weeks after lysimeter construction.

c = Determined by the EPA Methods for Chemical Analysis of Water and Wastes (1974).

d = Not Determined.

TABLE F-10. VOLATILE SOLIDS OF LEACHATES FROM THE UNIVERSITY OF CINCINNATI LYSIMETERS^a

Weeks ^b	Volatile Solids ^c (mg/l) of Leachates from Lysimeters:					
	UC-A	UC-B	UC-C	UC-D	UC-E	UC-F
4	1005	1246	1327	1327	ND ^d	ND
5	279	387	206	262	529	601
9	260	392	209	107	347	606
13	140	360	214	290	310	510
17	125	420	190	223	360	380
22	95	200	145	185	330	295
27	85	155	125	135	305	245
31	80	105	88	96	275	177
35	59	83	47	77	170	32
40	61	76	20	75	142	32
50	57	53	27	80	128	56

a = The lysimeter contents are: UC-A = Sewage Sludge

UC-B = Sewage Sludge + Municipal Solid Waste

UC-C = Municipal Solid Waste

UC-D = Municipal Solid Waste

UC-E = Hospital Waste + Municipal Solid Waste

UC-F = Hospital Waste

b = Weeks after lysimeter construction.

c = Determined by the EPA Methods for Chemical Analysis of Water and Wastes (1974)

d = Not Determined.

Reproduced from
best available copy.

